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(54) Title: SECRETED F-SPONDIN HOMOLOGS

(57) Abstract

The present invention relates to a secreted zsig25 polypeptide expressed at very high level in prostate tissue and polynucleotides encoding the same. The zsig25 polypeptides are believed to be adhesion-modulating and may be used for diagnosis of prostate adenocarcinoma or for sorting cancerous from non-cancerous cells. The present invention also includes antibodies to the zsig25 polypeptides.

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DESCRIPTION

SECRETED F-SPONDIN HOMOLOGS

BACKGROUND OF THE INVENTION

The gene encoding a secreted polypeptide designated F-spondin is expressed at high level in the rat floor plate. The floor plate is a cell group implicated in the control of neural cell pattern and axonal growth in the developing vertebrate nervous system. F-spondin is characterized by six repeats located in the C-terminal region. Such repeats were previously identified in thrombospondin and other molecules implicated in cell adhesion. Thus, F-spondin is believed to play a role in attachment of spinal cord and sensory neuron cells and the outgrowth of neurites. See, for example, US Patent No. 5,279,966.

The failure of tumor cell-tumor cell adhesion is believed to be a contributing factor to tumor metastases. See, for example, Zetter, Cancer Biology, 4: 219-29, 1993. Metastases, in turn, are generally associated with poor prognosis for cancer treatment. The metastatic process involves a variety of cellular events, including angiogenesis, tumor cell invasion of the vascular or lymphatic circulation, tumor cell arrest at a secondary site; tumor cell passage across the vessel wall into the parenchymal tissue, and tumor cell proliferation at the secondary site. Thus, both positive and negative regulation of adhesion are necessary for metastasis. That is, tumor cells must break away from the primary tumor mass, travel in circulation and adhere to cellular and/or extracellular matrix elements at a secondary site. Molecules capable of modulating cell-cell and cell-matrix adhesion are therefore sought for the study, diagnosis, prevention or treatment of metastases.

In 1990, prostate adenocarcinoma became the most frequent cancer diagnosed in American men. The peripheral zone of the prostate contains the bulk of the glandular component of the prostate and most prostate adenocarcinomas originate in this region. The peripheral zone consists of simple glands and loose stroma.

Generally, prostate cancer afflicts men over the age of 50 and intervention is dependent upon the stage to which the disease has progressed at the time of diagnosis. Stages A and B involve focal disease that is confined to the prostate, while stages C and D involve more disseminated disease. Indicia of stage C include expansion of the disease beyond the capsule with no regional lymph node involvement or more distant metastases. Stage D is associated with pelvic lymph node involvement or more distant metastases in lymph nodes, bone, lung or liver and the like. Stage A and B patients are commonly treated by radical prostatectomy, although radiation treatment may be used. Stage C patients are generally treated with radiation, because their disease has spread beyond the prostate and cannot be fully addressed by radical prostatectomy. Stage D patients are generally treated with hormone therapy (e.g., orchiectomy or chemotherapy).

In staging prostate cancer, understaging occurs more commonly than overstaging. Methods for staging prostate cancer include rectal examination, computed tomography and magnetic resonance imaging, pedal lymphangiography and fine needle biopsy guided by computed tomography. In addition, demonstration of prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) by immunoperoxidase staining of biopsy specimens can identify prostate cancer as the primary pathological site. These tumor markers are also found in the serum of prostate cancer patients and can be used for diagnosing prostate cancer and monitoring the response to treatment.

False negatives occur using the serum-based tests, however.

Consequently, markers are sought for prostate cancer or other solid tumors having metastatic potential, such as colon cancer, ovarian cancer and the like. Also, agents capable of reversing or blocking metastasis or capable of depressing elevated levels of prostate-cancer associated polypeptides are also sought for additional avenues of therapeutic intervention. Cell culture reagents useful in the study of metastases are also sought. Finally, cell culture reagents useful in assessing cell adhesion or proliferation are sought for use in research.

The present invention provides such polypeptides for these and other uses that should be apparent to those skilled in the art from the teachings herein.

SUMMARY OF THE INVENTION

Within one aspect, the present invention provides an isolated polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 27-277 of SEQ ID NO:2. Within one embodiment the polypeptide is at least 90% identical in amino acid sequence to residues 27-277 of SEQ ID NO:2. Within another embodiment the polypeptide comprises residues 1-277 of SEQ ID NO:2. Within another embodiment the polypeptide further comprises one thrombospondin type 1 domain carboxy terminal to the polypeptide. Within a related embodiment the thrombospondin type 1 domain comprises residues 278-330 of SEQ ID NO:2. Within further embodiments the polypeptide comprises residues 27-331 of SEQ ID NO:2. The polypeptide comprises residues 1-331 of SEQ ID NO:2. The polypeptide is from 251 to 331 amino acid residues in length. Within another embodiment the polypeptide is covalently linked amino terminally or carboxy terminally to a moiety

selected from the group consisting of affinity tags, toxins, radionucleotides, enzymes and fluorophores. Within a related embodiment the moiety is an affinity tag selected from the group consisting of polyhistidine, SEQ ID NO:20, Glu-Glu, glutathione S transferase and an immunoglobulin heavy chain constant region. Within a further related embodiment the polypeptide further comprises a proteolytic cleavage site between said sequence of amino acid residues and the affinity tag.

Within another aspect is provided a fusion protein consisting essentially of a first portion and a second portion joined by a peptide bond, the first portion comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 27-277 of SEQ ID NO:2; and the second portion consisting essentially of 2 to 8 thrombospondin type 1 domains. Within another aspect is provided a fusion protein comprising a secretory signal sequence having the amino acid sequence of amino acid residues 1-27 of SEQ ID NO:2, wherein the secretory signal sequence is operably linked to an additional polypeptide.

Within another aspect of the invention is provided an expression vector comprising the following operably linked elements: a transcription promoter; a DNA segment encoding a polypeptide as described above; and a transcription terminator. Within one embodiment the DNA segment encodes a polypeptide covalently linked to an affinity tag selected from the group consisting of polyhistidine, SEQ ID NO:20, Glu-Glu, glutathione S transferase and an immunoglobulin heavy chain constant region. Within another embodiment the DNA segment further encodes a secretory signal sequence operably linked to said polypeptide. Within a related embodiment the secretory signal sequence encodes residues 1-26 of SEQ ID NO:2. Also is provided a cultured cell into which has been introduced an expression vector as described above,

wherein the cell expresses the polypeptide encoded by the DNA segment.

Within another aspect is provided a method of producing a protein comprising: culturing a cell into which has been introduced an expression vector as described above, whereby the cell expresses the protein encoded by the DNA segment; and recovering the expressed protein.

Within other aspects the invention also provides a pharmaceutical composition comprising a polypeptide as described above in combination with a pharmaceutically acceptable vehicle. An antibody that specifically binds to an epitope of a polypeptide as described above. A binding protein that specifically binds to an epitope of a polypeptide as described above.

Within another aspect of the invention is provided an isolated polynucleotide encoding a polypeptide as described above. Within another embodiment the polynucleotide is from 830 to 1032 nucleotides in length. Within another embodiment the polynucleotide comprises nucleotide 1 to nucleotide 993 of SEQ ID NO:25. Within yet another embodiment, the polynucleotide is DNA.

Within another aspect the polynucleotide is selected from the group consisting of, a) a sequence of nucleotides from nucleotide 118 to nucleotide 870 of SEQ ID NO:1; b) a sequence of nucleotides from nucleotide 118 to nucleotide 1032 of SEQ ID NO:1; c) a sequence of nucleotides from nucleotide 40 to nucleotide 870 of SEQ ID NO:1; d) a sequence of nucleotides from nucleotide 40 to nucleotide 1032 of SEQ ID NO:1; e) orthologs of a), b), c) or d); f) allelic variants of a), b), c), d) or e); and g) nucleotide sequences complementary to a), b), c), d), e) or f).

Within another aspect is provided an isolated polynucleotide encoding a fusion protein consisting essentially of a first portion and a second portion joined

by a peptide bond, said first portion consisting essentially of a polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 27-277 of SEQ ID NO:2; and said second portion consisting essentially of 2 to 8 thrombospondin type 1 domains. Within another aspect is provided an isolated polypeptide encoding a fusion protein comprising a secretory signal sequence having the amino acid sequence of amino acid residues 1-27 of SEQ ID NO:2, wherein said secretory signal sequence is operably linked to an additional polypeptide.

Within yet another embodiment is provided an oligonucleotide probe or primer comprising at least 14 contiguous nucleotides of a polynucleotide of SEQ ID NO:25 or a sequence complementary to SEQ ID NO:25.

The invention also provides a method for detecting a genetic abnormality in a patient, comprising: obtaining a genetic sample from a patient; incubating the genetic sample with a polynucleotide comprising at least 14 contiguous nucleotides of SEQ ID NO:1 or the complement of SEQ ID NO:1, under conditions wherein the polynucleotide will hybridize to complementary polynucleotide sequence, to produce a first reaction product; comparing the first reaction product to a control reaction product, wherein a difference between the first reaction product and the control reaction product is indicative of a genetic abnormality in the patient.

These and other aspects of the invention will become evident upon reference to the following detailed description of the invention and the attached drawing(s).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates an alignment of *Rattus norvegicus* F-spondin secreted protein (FSPO_R) and a zsig25 polypeptide of the present invention.

Figure 2 schematically shows a domain level alignment of TSP1 (Lawler and Hynes, J. Cell Biol. 103: 1635-48, 1986), TSP2 (Labell and Byers, Genomics 17: 225-9, 1993), FSPO_R (Klar et al., Cell 69: 95-110, 1992), FSPO_xenla (Altaba et al., Proc. Natl. Acad. Sci. USA 90: 8268-72, 1993), CELF10E7_7 (Nature 368(6446): 32-8, 1994), and a zsig25 polypeptide of the present invention, wherein Hep bind connotes a heparin binding domain; VFWC connotes a Von Willebrand Factor type C repeat (Mancuso et al., J. Biol. Chem. 264: 19514-27, 1989); TSP-1 connotes a thrombospondin type 1 domain and the number outside the parentheses indicates the number of repeats; EGF-1 connotes an Epidermal Growth Factor Type I repeat and the number outside the parentheses indicates the number of repeats; TSP-3 connotes a thrombospondin type 3 domain and the number outside the parentheses indicates the number of repeats; RR connotes an arginine-arginine sequence; Kunitz connotes a kunitz domain and TFP-1 like connotes a domain having features similar to that of a thrombospondin type 1 domain.

Figure 3 Shows a dose response in BaF3 pZR103#2 cells to zsig25NF.

Figure 4 Shows a dose response in DA-1 cells to zsig25NF.

Figure 5 Shows a dose response in 1° marrow cells with murine IL-3.

Figure 6 Shows a dose response in 1° marrow cells to zsig25NF with and with out murine IL-7.

Figure 7 Shows platelet count in myelosuppressed mice administered adenovirus expressing zsig25 NF, zsig25 CF, empty virus and an irradiated only control.

Figure 8 Shows a hydrophilicity profile for amino acid sequence (SEQ ID NO:2) of zsig25.

DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention in detail, it may be helpful to the understanding thereof to define the following terms:

The term "affinity tag" is used herein to denote a peptide segment that can be attached to a polypeptide to provide for purification or detection of the polypeptide or provide sites for attachment of the polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al., Methods Enzymol. 198:3, 1991), glutathione S transferase (Smith and Johnson, Gene 67:31, 1988), Glu-Glu affinity tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA 82:7952-4, 1985), substance P, Flag™ peptide (Hopp et al., Biotechnology 6:1204-10, 1988; available from Eastman Kodak Co., New Haven, CT), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general Ford et al., Protein Expression and Purification 2: 95-107, 1991. DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

The term "allelic variant" denotes any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides and proteins. Where the context allows, these terms are used with reference to a particular

sequence or portion of a polypeptide or protein to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a protein is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete protein.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of $<10^9$ M^{-1}.

The term "complements of polynucleotide molecules" denotes polynucleotide molecules having a complementary base sequence and reverse orientation as compared to a reference sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a polynucleotide that has a contiguous stretch of identical or complementary sequence to another polynucleotide. Contiguous sequences are said to "overlap" a given stretch of polynucleotide sequence either in their entirety or along a partial stretch of the polynucleotide. For example, representative contigs to the polynucleotide sequence 5'-ATGGCTTAGCTT-3' are 5'-TAGCTTgagtct-3' and 3'-gtcgacTACCGA-5'.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or

more degenerate codons (as compared to a reference polynucleotide molecule that encodes a polypeptide). Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

The term "expression vector" denotes a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments may include promoter and terminator sequences, and may optionally include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

The term "isolated", when applied to a polynucleotide molecule, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, Nature 316:774-78, 1985). When applied to a protein, the term "isolated" indicates that the protein is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated protein is substantially free of other proteins, particularly other proteins of animal origin. It is preferred to provide the protein in a

highly purified form, i.e., greater than 95% pure, more preferably greater than 99% pure.

The term "operably linked", when referring to DNA segments, denotes that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in the promoter and proceeds through the coding segment to the terminator.

The term "ortholog" (or "species homolog") denotes a polypeptide or protein obtained from one species that has homology to an analogous polypeptide or protein from a different species. The ortholog is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α-globin, β-globin, and myoglobin are paralogs of each other.

The term "polynucleotide" denotes a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules. Sizes of polynucleotides are expressed as base pairs (abbreviated "bp"), nucleotides ("nt"), or kilobases ("kb"). Where the context allows, the latter two terms may describe polynucleotides that are single-stranded or double-stranded. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term "base pairs". It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide may differ slightly in length and that the ends thereof may be staggered as a result of enzymatic cleavage; thus all nucleotides within a double-stranded polynucleotide

molecule may not be paired. Such unpaired ends will in general not exceed 20 nt in length.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides".

"Probes and/or primers" as used herein can be RNA or DNA. DNA can be either cDNA or genomic DNA. Polynucleotide probes and primers are single or double-stranded DNA or RNA, generally synthetic oligonucleotides, but may be generated from cloned cDNA or genomic sequences or its complements. Analytical probes will generally be at least 20 nucleotides in length, although somewhat shorter probes (14-17 nucleotides) can be used. PCR primers are at least 5 nucleotides in length, preferably 15 or more nt, more preferably 20-30 nt. Short polynucleotides can be used when a small region of the gene is targeted for analysis. For gross analysis of genes, a polynucleotide probe may comprise an entire exon or more. Probes can be labeled to provide a detectable signal, such as with an enzyme, biotin, a radionuclide, fluorophore, chemiluminescer, paramagnetic particle and the like, which are commercially available from many sources, such as Molecular Probes, Inc., Eugene, OR, and Amersham Corp., Arlington Heights, IL, using techniques that are well known in the art.

The term "promoter" denotes a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be

added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule (i.e., a ligand) and mediates the effect of the ligand on the cell. Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. Binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell. This interaction in turn leads to an alteration in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. Most nuclear receptors also exhibit a multi-domain structure, including an amino-terminal, transactivating domain, a DNA binding domain and a ligand binding domain. In general, receptors can be membrane bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor).

The term "secretory signal sequence" denotes a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger peptide

is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein encoded by a splice variant of an mRNA transcribed from a gene.

Molecular weights and lengths of polymers determined by imprecise analytical methods (e.g., gel electrophoresis) will be understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

The present invention is based in part upon the discovery of novel DNA sequences (SEQ ID NOS. 1 and 3) and a corresponding polypeptide (SEQ ID NO. 2) having homology to F-spondin expressed selectively by the rat floor plate and believed to be involved in floor plate and neural development (SEQ ID NO. 4). See, for example, Klar et al., Cell 69: 95-110, 1992. According to the Klar article, rat floor plate F-spondin is characterized by an amino-terminal region having no known homology and a carboxy-terminal region having six terminal thrombospondin type 1 repeats. The zsig25 polypeptides of the present invention are homologous to the amino-terminal region of rat floor plate F-spondin.

Analysis of the tissue distribution of the mRNA corresponding to this novel DNA by Northern blot and Dot blot analysis showed that expression was highest in prostate, lower in placenta, ovary, small intestine and

peripheral blood leukocytes, and apparent but decreased in testis, heart, adrenal gland and colon. Two transcript sizes were observed, one at approximately 2 kb and one at approximately 5 kb. The 2 kb message was detected in much higher abundance than the 5 kb. The polynucleotide sequences in SEQ ID NOS. 1 and 3 appear to be encompassed in the 2 kb message. Additional analysis showed a single 2.2 kb or a 2.2 kb and 6.0 kb transcript in fetal lung, liver, kidney, muscle, heart, skin and small intestine. A 2 kb transcript was also detected in $CD4^+$ and $CD8^+$ cells. The polypeptide of the present invention has been designated zsig25.

The novel zsig25 polypeptide-encoding polynucleotides of the present invention were initially identified by querying an EST database for secretory signal sequences characterized by an upstream methionine start site, a hydrophobic region of approximately 13 amino acids and a cleavage site (SEQ ID NO. 5, wherein cleavage occurs between the glycine and glutamine amino acid residues) in an effort to select for secreted proteins. ESTs meeting those search criteria were compared to known sequences to identify secreted proteins having homology to known ligands.

Two EST sequences were discovered and were determined to be novel and predicted to be related to a secreted F-spondin protein found in rats based upon homology. See, for example, Klar et al., ibid. and Figures 1 and 2. One EST, designated zsig25a clone, was from a human uterine tissue library, is a 1354 bp cDNA including an Eco RI linker and an oligonucleotide dT priming site. It is believed that this EST was generated by a non-specific priming event. The second EST, also derived from a human breast tumor tissue library and designated zsig25b clone, is a 1446 bp cDNA containing a polyadenylation sequence downstream of a putative polyadenylation signal. The zsig25a and zsig25b clones were found to overlap for

1137 bases, sharing 100% identity in the overlapping region. Zsig25a was 188 bp longer on the 5' end and contains the putative signal sequence, while zsig25b was 280 bp longer on the 3' end and contained the putative polyadenylation signal. The resulting full length composite sequence is the 1607 bp sequence shown in SEQ ID NO. 1.

Contemporaneously, a human fetal heart library was examined in an effort to select polypeptides with interesting homology. A polypeptide having an overlapping region to a clone identified above was also identified as having homology to F-spondin. A search for contigs relating to the selected polypeptide revealed 9 contigs in prostate and 1 contig in white blood cells. Other libraries that might also be searched for such clones include uterus, prostate, breast, placenta, ovary, small intestine, testis, heart, adrenal gland, colon and the like.

The deduced amino acid sequence (SEQ ID NO. 2; Met at position 1) is encoded by the polynucleotides of SEQ ID NO. 1. Analysis of the DNA encoding a zsig25 polypeptide (SEQ ID NO. 1) revealed an open reading frame encoding 331 amino acids (SEQ ID NO. 2) comprising a 5' untranslated region; a secretory signal peptide of 26 amino acid residues (residue 1 (Met) to residue 26 (Gly) of SEQ ID NO. 2) and a mature polypeptide of 307 amino acids (residue 27 (Gln) to residue 331 (Val) of SEQ ID NO. 2). N-glycosylation of the zsig25 polypeptide may occur at putative N-glycosylation sites located at amino acid residues 48-50 and 280-282 of SEQ ID NO. 2. Those skilled in the art will recognize that predicted secretory signal sequence domain boundaries are approximations based on primary sequence content, and may vary slightly; however, such estimates are generally accurate to within ± 4 amino acid residues. Therefore the present invention also includes the polypeptides having amino acid sequences

comprising amino acid residues 23-331 of SEQ ID NO:2, residues 24-331 of SEQ ID NO:2, residues 25-331 of SEQ ID NO:2, residues 26-331 of SEQ ID NO:2, residues 27-331 of SEQ ID NO:2, residues 28-331 of SEQ ID NO:2, residues 29-331 of SEQ ID NO:2 and residues 30-331 of SEQ ID NO:2 as well as the polynucleotides encoding them.

Zsig25 polypeptides are characterized by an amino terminal-domain having homology to rat floor plate F-spondin and a truncated carboxy terminal domain that appears to be a single thrombospondin type 1 domain. Thrombospondin type I domains are repeating domains found in thrombospondin and other proteins that are believed to be involved in adhesion. Thrombospondin type 1 domains are known in the art, and rat F-spondin is known to have six such domains at the carboxy terminal end thereof. Also, rat F-spondin is described in the literature as having no known homology in the amino-terminal domain.

R. norvegicus F-spondin protein (FSPO_R) is an 807 amino acid protein (SEQ ID NO. 3; Met is at position 1), having a carboxy-terminal portion composed of six thrombospondin repeat (TSR) sequences. The amino-terminal portion of FSPO_R comprises amino acid residues ranging from 1 to 440 and includes 10 cysteine residues. The carboxy-terminal portion of FSPO_R ranges from amino acid residue 441 to 807 (Klar et al., ibid.). Alignment of zsig25 with FSPO_R, as shown in Figure 1, revealed a significant percent identity in the N-terminal domain of the polypeptides corresponding to the region from amino acid residues 35 (Cys) to amino acid residue 278 (Cys) of SEQ ID NO. 2. This conserved N-terminal region may connote a new subfamily of proteins.

Within the N-terminal region of high identity, the following percent identity figures are observed for the deduced amino acid sequence of SEQ ID NO. 2 and rat F-spondin (SEQ ID NO. 3).

	Zsig25	Rat F-spondin
Zsig25	100	35
Rat F-spondin	35	100

Given the predicted secretory signal sequence, the amino-terminal domain of the zsig25 polypeptide of SEQ ID NO. 2 appears to be about 150 amino acids shorter than that of rat F-spondin. Also, the carboxy-terminal domain of the zsig25 polypeptide of SEQ ID NO. 2 appears to be truncated as well, apparently containing a single TSR. The alignment also revealed that the two polypeptides shared putative N-glycosylation sites at amino acid residues 48-50 and 280-282 and cysteine residues at position 15, 35 and 169 in the amino-terminal domain and at positions 276, 287 and 292 in the carboxy-terminal domain, wherein the residue numbers correspond to those shown in the Fig. 1 for zsig25 polypeptide and in SEQ ID NO. 2.

The highly conserved amino acids, both within and without the region of high identity, can be used as a tool to identify zsig25 polypeptides or zsig25-like proteins. For instance, reverse transcription-polymerase chain reaction (RT-PCR) can be used to amplify sequences encoding the conserved motifs suggested by the multiple alignment from RNA obtained from a variety of tissue sources. In particular, the following primers are useful for this purpose:

zsig25 residues 38-43

degenerate: GN GCN CCN GCN AAR TAY (SEQ ID NO:43)
consensus: GN GSN MCN GCN AAR TAY (SEQ ID NO:44)
complement: CN CSN KGN CGN TTY ART (SEQ ID NO:45)

zsig25 residues 46-52

degenerate: CAN TTY CAN GGN AAR TG (SEQ ID NO:46)
consensus: CAN TTY WMN GGN AAN TG (SEQ ID NO:47)
complement: TGN AAR WKN CCN TTN AC (SEQ ID NO:48)

zsig25 residues 56-61

degenerate	TTY CCN AAR CAR TAY CC	(SEQ ID NO:49)
consensus:	YWY CCN AAR SAN TAY CC	(SEQ ID NO:50)
complement:	RWR GGN TTY STN ART GG	(SEQ ID NO:51)

zsig25 residues 159-164

degenerate:	CCN GAY TTG TTY GTN GG	(SEQ ID NO:52)
consensus:	CCN DAY TGG WWY GTN GG	(SEQ ID NO:53)
complement:	GGN CTR ACC WWR CAN CC	(SEQ ID NO:54)

zsig25 residues 189-191

degenerate:	CCN TAY GAY GCN GGN AC	(SEQ ID NO:55)
consensus:	CCN TRB GAY GCN GGN AC	(SEQ ID NO:56)
complement:	GGN AYV CRT CGN CCN TG	(SEQ ID NO:57)

zsig25 residues 203-208

degenerate:	GCN ANC ATH CCN CAR GA	(SEQ ID NO:58)
consensus:	SCN CAN ATH CCN CAR GA	(SEQ ID NO:59)
complement:	GGN AYV CRT CGN CCN TG	(SEQ ID NO:60)

Fig. 2 schematically shows the domain structure of the zsig25 polypeptides of the present invention, two F-spondin proteins (FSPO_rat and FSPO_xenla), two thrombospondin proteins (TSP1 and TSP2) and another protein having homology to the N-terminal region of F-spondin (CELF10E7_7). The brief description of Fig. 2 includes the key for the domain abbreviations employed below. TSP1 and TSP2 share the following basic structure: Hep bind domain, VWFC domain, three tandem TSP-1 domains, three tandem EGF-1 domains and three tandem TSP-3 domains. CELF10E7_7 includes an F-spondin-like N-terminal domain, three tandem TSP-1 domains, an arginine-arginine (RR) sequence (a potential dibasic post-processing site which may be cleaved by a number of activating/deactivating enzymes), a Kunitz domain and a TSP-1 domain. FSPO_rat

and FSPO_xenla share the following basic structure: an F-spondin N-terminal domain and six tandem TSP-1 domains. Zsig25 polypeptides of the present invention incorporate a truncated F-spondin N-terminal domain and a single TSP-1 domain.

The present invention also provides polynucleotide molecules, including DNA and RNA molecules, that encode the zsig25 polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. SEQ ID NO:25 is a degenerate DNA sequence that encompasses all DNAs that encode the zsig25 polypeptide of SEQ ID NO:2. Those skilled in the art will recognize that the degenerate sequence of SEQ ID NO:25 also provides all RNA sequences encoding SEQ ID NO:2 by substituting U for T. Thus, zsig25 polypeptide-encoding polynucleotides comprising nucleotide 1 to nucleotide 993 of SEQ ID NO:25 and their RNA equivalents are contemplated by the present invention. Table 1 sets forth the one-letter codes used within SEQ ID NO:25 to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.

TABLE 1

Nucleotide	Resolution	Nucleotide	Complement
A	A	T	T
C	C	G	G
G	G	C	C
T	T	A	A
R	A|G	Y	C|T
Y	C|T	R	A|G
M	A|C	K	G|T
K	G|T	M	A|C
S	C|G	S	C|G
W	A|T	W	A|T
H	A|C|T	D	A|G|T
B	C|G|T	V	A|C|G
V	A|C|G	B	C|G|T
D	A|G|T	H	A|C|T
N	A|C|G|T	N	A|C|G|T

The degenerate codons used in SEQ ID NO:25, encompassing all possible codons for a given amino acid, are set forth in Table 2.

TABLE 2

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn|Asp	B		RAY
Glu|Gln	Z		SAR
Any	X		NNN

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding each amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequence of SEQ ID NO:2. Variant sequences can be readily tested for functionality as described herein.

One of ordinary skill in the art will also appreciate that different species can exhibit "preferential codon usage." In general, see, Grantham, et al., Nuc. Acids Res. 8:1893-912, 1980; Haas, et al. Curr. Biol. 6:315-24, 1996; Wain-Hobson, et al., Gene 13:355-64, 1981; Grosjean and Fiers, Gene 18:199-209, 1982; Holm, Nuc. Acids Res. 14:3075-87, 1986; Ikemura, J. Mol. Biol. 158:573-97, 1982. As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (See Table 2). For example, the amino acid Threonine (Thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different Thr codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential

codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequence disclosed in SEQ ID NO:25 serves as a template for optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

Radiation hybrid mapping is a somatic cell genetic technique developed for constructing high-resolution, contiguous maps of mammalian chromosomes (Cox et al., Science 250:245-250, 1990). Partial or full knowledge of a gene's sequence allows the designing of PCR primers suitable for use with chromosomal radiation hybrid mapping panels. Radiation hybrid mapping panels are commercially available which cover the entire human genome, such as the Stanford G3 RH Panel and the GeneBridge 4 RH Panel (Research Genetics, Inc., Huntsville, AL). These panels enable rapid, PCR based, chromosomal localizations and ordering of genes, sequence-tagged sites (STSs), and other nonpolymorphic- and polymorphic markers within a region of interest. This includes establishing directly proportional physical distances between newly discovered genes of interest and previously mapped markers. The precise knowledge of a gene's position can be useful in a number of ways including: 1) determining if a sequence is part of an existing contig and obtaining additional surrounding genetic sequences in various forms such as YAC-, BAC- or cDNA clones, 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region, and 3) for cross-referencing model organisms such as mouse which may be beneficial in helping to determine what function a particular gene might have.

Radiation hybrid mapping showed that the gene encoding the zsig25 polypeptide maps 15.23 cR_3000 from the top of the human chromosome 4 linkage group on the WICGR radiation hybrid map. Proximal and distal framework markers were WI-6657 (D4S2799) and WI-5430 (D4S2663), respectively. This positions the zsig25 gene in the 4p16.3 region on the integrated LDB chromosome 4 map (The Genetic Location Database, University of Southhampton, WWW server: http://cedar.genetics. soton.ac.uk/public_html/).

Of particular interest to note is that the critical region for the Wolf-Hirschhorn syndrome has been mapped to this region (Wright et al., Hum. Mol. Genet. 6: 317-24, 1997). Wolf-Hirschhorn syndrome is characterized by a deletion of a segment of one chromosome 4 short arm at 4p16.3. The article describes a transcript map of the newly defined 165 kb Wolf-Hirschhorn syndrome critical region. Heart defects are often observed in connection with Wolf-Hirschhorn syndrome, which observation is consistent with the expression of zsig25 polypeptide in the heart. Wolf-Hirschhorn syndrome is characterized by developmental and mental defects. Zsig25 plays a role in cellular proliferation and differentiation and therefor deletion of such a gene would likely lead to developmental defects. Thus, the present invention provides reagents which may find use in the diagnosis and/or treatment of Wolf-Hirschhorn syndrome.

The present invention provides reagents for use in diagnostic applications. For example, the zsig25 gene, a probe comprising zsig25 DNA or RNA, or a subsequence thereof can be used to determine if the zsig25 gene is present on chromosome 4 or if a mutation has occurred. Detectable chromosomal aberrations at the zsig25 gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. These aberrations can occur within the coding sequence, within introns, or

within flanking sequences, including upstream promoter and regulatory regions, and may be manifested as physical alterations within a coding sequence or changes in gene expression level.

In general, these diagnostic methods comprise the steps of (a) obtaining a genetic sample from a patient; (b) incubating the genetic sample with a polynucleotide probe or primer as disclosed above, under conditions wherein the polynucleotide will hybridize to complementary polynucleotide sequence, to produce a first reaction product; and (iii) comparing the first reaction product to a control reaction product. A difference between the first reaction product and the control reaction product is indicative of a genetic abnormality in the patient. Genetic samples for use within the present invention include genomic DNA, cDNA, and RNA. The polynucleotide probe or primer can be RNA or DNA, and will comprise a portion of SEQ ID NO:1, the complement of SEQ ID NO:1, or an RNA equivalent thereof. Suitable assay methods in this regard include molecular genetic techniques known to those in the art, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, ligation chain reaction (Barany, PCR Methods and Applications 1:5-16, 1991), ribonuclease protection assays, and other genetic linkage analysis techniques known in the art (Sambrook et al., ibid.; Ausubel et. al., ibid.; Marian, Chest 108:255-65, 1995). Ribonuclease protection assays (see, e.g., Ausubel et al., ibid., ch. 4) comprise the hybridization of an RNA probe to a patient RNA sample, after which the reaction product (RNA-RNA hybrid) is exposed to RNase. Hybridized regions of the RNA are protected from digestion. Within PCR assays, a patient's genetic sample is incubated with a pair of polynucleotide primers, and the region between the primers is amplified and recovered. Changes in size or amount of recovered

product are indicative of mutations in the patient. Another PCR-based technique that can be employed is single strand conformational polymorphism (SSCP) analysis (Hayashi, PCR Methods and Applications 1:34-8, 1991).

Zsig25 polypeptides are found in human prostate tissue in high abundance. Thus, zsig25 polypeptides are believed to be associated with prostate function. Zsig25 polypeptides also share homology with F-spondin, a polypeptide implicated in cell adhesion. Thus, zsig25 polypeptides, fragments, fusions, agonists or antagonists are believed to be useful as *in vitro* culture reagents to study homotypic or heterotypic adhesion. Methods of modulating cell-cell, cell-platelet or cell-extracellular matrix adhesion in cell culture in accordance with the present invention comprise incubating eukaryotic cells in a culture medium including a zsig25 polypeptide, fragment, fusion, agonist or antagonist and comparing observed adhesion with that observed when the cells are incubated with a medium alone. Homotypic (cell-like cell) adhesion may be evaluated using standard techniques, such as aggregometry (by change in light transmission), light microscopy, gamma scintigraphy using chromium-labeled cell lines, and spectrofluorimetry using 2/, 7/-bis (2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-labeled cell lines. Heterotypic (cell-platelet, cell-extracellular matrix or cell-unlike cell) adhesion may be evaluated using transmigration assays, ELISA-format assays and the like. Assays to assess metastatic potential, assessed using adhesion parameters, are known in the art. See, for example, Koenigsmann et al., Onkologie 17: 528-37, 1994, Asao et al., Cancer Letters 78: 57-62, 1994 and the like.

In a preferred embodiment of this aspect of the present invention, the cells studied are prostate cells or prostate cancer cells. Some useful cell lines for this preferred adhesion function evaluation can be obtained from the ATCC, including ATCC CRL-1740 (metastatic

prostate adenocarcinoma, human), ATCC CRL-1435 (prostate adenocarcinoma, human), ATCC CRL-2220 (adenocarcinoma, prostate, HPV-18 transfected, human), ATCC HTB-81 (carcinoma, prostate, metastasis to brain, human), ATCC CRL-2221 (normal prostate, HPV-18 transfected, human), ATCC CRL-2098 (primitive multipotential sarcoma, bone, human), ATCC CRL-5813 (prostate, small cell carcinoma, human) and the like. Also, primary tissue samples may be employed in such evaluations.

Zsig25 was found to stimulate the proliferation of hematopoietic cells, in particular, BaF3 and DA-1 cells, as described in detail below. BaF3 is an interleukin-3 dependent pre-lymphoid cell line derived from murine bone marrow. DA-1 is an IL-3 dependent cell line derived from the lymph node of a mouse with a B-cell lymphoma. Thus, zsig25 polypeptides, fragments, fusions, agonists or antagonists are believed to be associated with the production of B-cells from the bone marrow. Specifically, zsig25 polypeptides could be used to enhance the production of B-cells from bone marrow to bolster humoral immunity. Such an application could be made during or following bone marrow transplantation or other application where stimulation of B-cell development is desired. Zsig25 polypeptides may be used in association with other bone marrow stimulating proteins, such as thrombopoietin or erythropoietin to enhance recovery following transplantation. The invention provides methods of stimulating B-cell proliferation by administering to a patient in need of such treatment, a pharmaceutically effective amount of a polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 27-277 of SEQ ID NO:2.

Antibodies to zsig25 could be used to neutralize the effects of native zsig25 for treating pre-B or B-cell leukemias, such as plasma cell leukemia, chronic or acute lymphocytic leukemia; myelomas such as multiple myeloma,

plasma cell myeloma, endothelial myeloma and giant cell myeloma; and lymphomas such as non-Hodgkins lymphoma, that produce zsig25 polypeptides. Northern blot analysis showed zsig25 expression in $CD8^+$ cells. This suggests that in some autoimmune disorders, cytotoxic T-cells might stimulate B-cell production through excess production of zsig25. Antibodies or antagonists to zsig25 could be used to neutralize the effects of zsig25 in these autoimmune diseases.

Zsig25 polypeptides would be of therapeutic value in treating immunodeficiencies. X-linked agammaglobulinemia (XLA) is a congenital antibody deficiency disease linked to the X chromosome (Rosen et al., New England J. Med.333:431-9, 1995). Patients with XLA have mutations in the Burton's tyrosine kinase gene. X-linked immunodificient (xid) mice share this mutation (Brorson et al., J. Immunol. 159:135-43, 1997). The major phenotype associated with XLA is a complete or near complete arrest of B lymphoid development between the pre-B-cell stage and mature B-cells. Patients with XLA lack B-cells and associated humoral immunity. Some patients and xid mice display a "leaky" phenotype, in that some B-cells mature and mount limited humoral immune responses. This suggests that other pathways exist for B-cell development. Zsig25 acts on B-cells at this transition point. Application may be made of zsig25 to stimulate B-cell development and restore partial or full humoral immunity to XLA patients. The xid mouse would serve as a model for XLA and could be used for *in vivo* testing of zsig25 polypeptides. Xid mouse cells would be useful for *in vitro* assays as well. Zsig25 can be used independently or in conjunction with cytokines which have been demonstrated to transform B-cells into efficient APC for presentation of antigen to T-cells (Zimecki and Kapp, Arch. Immunol. Ther. Exper. 43:253-7, 1995).

Another immunodeficiency associated with B-cell development is common variable immunodeficiency (CVID) which encompasses several immune deficiency diseases. Zsig25 polypeptides and polynucleotides disclosed herein can serve as a diagnostic tools for identifying and monitoring subsets of those patients exhibiting zsig25 deficiency.

Zsig25 C-terminally-FLAG tagged polypeptides were found to significantly reduce the platelet recovery time in myelosuppressed mice as described in detail below. Megakaryocyte hyperplasia and increased platelet counts (thrombocytosis) are associated with a variety of diseases, such as trauma, iron deficiency anemia, acute and chronic infection, various solid tumors, myeloproliferative disorders and to a lesser extent myelodisplastic disorders. Conditions of thrombocytosis occur following splenectomy and other surgical procedures. Elevated platelet counts are a common pediatric occurrence. Persistent thrombocytosis associated with myeloproliferative disorders is associated with an increased frequency of venous thromboembolism. Lowering platelet counts provides clinical benefit to patients with thrombocytosis and active bleeding or thrombosis. Patients who are at risk of thromboembolic complications associated with myeloproliferative disorders and microvascular occlusive syndromes, characterized by digital or cerebral ischemia, benefit from a lowering of platelet counts. Thus, zsig25 polypeptides, peptides, fragments and agonists thereof would be of therapeutic value in lowering platelet levels in patients requiring such treatment. The invention provides methods of alleviating thrombocytosis by administering to a patient in need of such treatment, a pharmaceutically effective amount of a polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 27-277 of SEQ ID NO:2. Additionally,

cellular adhesive properties of zsig25 polypeptides may be associated with platelet aggregation.

The zsig25 polypeptides, fragments, fusions, agonists or antagonists of the present invention may also prove useful in the study of proliferation, differentiation or cell signaling, e.g., of prostate cells or prostate cancer cells, or hematopoietic cells, e.g., B-cells, platelets. General methods modulating the proliferation or cell signaling of cells in cell culture comprise incubating eukaryotic cells in a culture medium including a zsig25 polypeptide, fragment, fusion, agonist or antagonist and comparing observed proliferation or cell signaling with that observed when the cells are incubated with medium alone. Useful cell proliferation/metabolism assays for this purpose are known in the art. Mitogenic activity can be measured using known assays, including ^{3}H-thymidine incorporation assays (as disclosed by, e.g., Raines and Ross, Methods Enzymol. 109:749-73, 1985), dye incorporation assays (as disclosed by, for example, Mosman, J. Immunol. Meth. 65:55-63, 1983 and Raz et al., Acta Trop. 68:139-47, 1997) or cell counts. Cell signaling can, for example, be evaluated using known assays, such as reporter gene-based assays. Such assays involve the use of reporter genes, e.g., a serum responsive element-detectable protein encoding gene construct. The cell lines set forth above and other cell lines known in the art are useful for these proliferation and cell signaling assays. Additional assays for measuring proliferation include such assays as chemosensitivity to neutral red dye (Cavanaugh et al., Investigational New Drugs 8:347-354, 1990, incorporated herein by reference), incorporation of radiolabelled nucleotides (Cook et al., Anal. Biochem. 179:1-7, 1989, incorporated herein by reference), incorporation of 5-bromo-2'-deoxyuridine (BrdU) in the DNA of proliferating cells (Porstmann et al., J. Immunol. Meth. 82:169-179,

1985, incorporated herein by reference), and use of tetrazolium salts (Mosmann, J. Immunol. Meth. 65:55-63, 1983; Alley et al., Cancer Res. 48:589-601, 1988; Marshall et al., Growth Reg. 5:69-84, 1995; and Scudiero et al., Cancer Res. 48:4827-33, 1988). Assays measuring differentiation include, for example, measuring cell-surface markers associated with stage-specific expression of a tissue, enzymatic activity, functional activity or morphological changes (Watt, FASEB, 5:281-4, 1991; Francis, Differentiation 57:63-75, 1994; Raes, Adv. Anim. Cell Biol. Technol. Bioprocesses, 161-71, 1989).

Rat F-spondin has been shown to stimulate nerve cell growth and adhesion. Useful adhesion assays and neurite cell growth assays are known to those skilled in the art. See, for example, US Patent No. 5,279,966.

Hepatocellular tissue damage in the liver can result from inflammation caused by an immune response such as in viral hepatitis. Administration of adenovirus into a mouse normally results in some degree of liver inflammation. There was a significant inhibition of inflammation in the livers of myelosuppressed mice having an adenovirus expressing a zsig25NF polypeptide as compared to mice having adenovirus expressing zsig25CF and null polypeptides, which is described in greater detail below. Liver enzymes, ALT and AST, were higher in the virus treated mice when compared to the untreated controls. ALT levels were higher in the AdCMV-null and AdCMV-zsig-25CF treated mice when compared to the AdCMV-zsig-25NF treated mice which correlated with the adenovirus dosage given to the mice. Zsig25 polypeptides are therefore considered for applications where it is desired to reduce a inflammatory response in the liver. This would include stimulating a reduction in the number and activity of inflammatory cells in the liver, and/or diminishing inflammation of the liver. Such anti-inflammatory polypeptides would find application in the

treatment of viral hepatitis, including viral hepatitis A, B, C, D and E. Also included are other acute hepatitis syndromes caused by Epstein-Barr virus, cytomegalovirus, herpes simplex virus, yellow fever and rubella. Additionally are liver inflammations induced by toxins or drugs as well as parasitic, bacterial, fungal or granulomatous associated liver diseases. Zsig25 polypeptides would also be useful for alleviation of inflammation associated with the various forms of chronic hepatitis. The invention provides methods of reducing an inflammatory response in the liver by administering to a patient in need of such treatment a pharmaceutically effective amount of a polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 27-277 of SEQ ID NO:2.

The present invention also involves the use of the zsig25 polypeptides in cell sorting applications. More specifically zsig25 polypeptides are affixed to a solid support, such as magnetic beads. Such immobilized zsig25 may be useful in cell sorting procedures, such as to sort cancerous from non-cancerous cells, using fluorescence-activated cell sorting, for example. In a preferred embodiment of this aspect of the present invention, the cells are prostate cells or prostate cancer cells.

A diagnostic method of the present invention involves the detection of zsig25 polypeptides in the serum or tissue biopsy of a patient undergoing evaluation for possible prostate cancer or pre B-cell or B-cell leukemias, myelomas or lymphomas. Such zsig25 polypeptides can be detected using immunoassay techniques and antibodies capable of recognizing a zsig25 polypeptide epitope. More specifically, the present invention contemplates methods for detecting zsig25 polypeptide comprising:

exposing a solution possibly containing zsig25 polypeptide to an antibody attached to a solid support, wherein said antibody binds to a first epitope of a zsig25 polypeptide;

washing said immobilized antibody-polypeptide to remove unbound contaminants;

exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a zsig25 polypeptide, wherein the second antibody is associated with a detectable label; and

detecting the detectable label. Levels of zsig25 polypeptide in serum or biopsy zsig25 polypeptide appear to be indicative of adenocarcinoma of the prostate or pre-B-cell or B-cell disorders.

Other aspects of the present invention involve the study of and treatment of metastatic disorders. As a preliminary matter, zsig25 polypeptides, fragments, fusions agonists or antagonists thereof will be evaluated to determine whether zsig25 polypeptides modulate tumor cell-tumor cell association, tumor cell-platelet, tumor cell-epithelial cell association or tumor cell-extracellular matrix association. Molecules which enhance or upregulate tumor cell-tumor cell association will have utility in prevention of metastasis, as will molecules which diminish or down-regulate tumor cell-platelet, tumor cell-epithelial cell association or tumor cell-extracellular matrix association. Moieties exhibiting both enhanced homotypic aggregation and depressed heterotypic aggregation are more preferred. The adhesion assays referenced above would be useful in these aspects of the present invention as well.

Also, serum levels of molecules which diminish or down-regulate tumor cell-tumor cell association are expected to have predictive value with respect to the potential for tumor metastasis, as are serum levels of molecules which enhance or upregulate tumor cell-platelet,

tumor cell-epithelial cell or tumor cell-extracellular matrix association. The immunoassays described above would also be useful in these diagnostic aspects of the present invention.

Agonists or antagonists of the zsig25 polypeptides disclosed above are included within the scope of the present invention. Agonists may be identified using a method that comprises providing cells responsive to a zsig25 polypeptide, fragment or fusion, culturing the cells in the presence of a test compound and comparing the cellular response with the cell cultured in the presence of the zsig25 polypeptide, and selecting the test compounds for which the cellular response is of the same type. Useful agonists of zsig25 polypeptide can also include anti-idiotypic antibodies raised against antibodies recognizing zsig25 polypeptides.

Antagonists may be identified by a method that comprises providing cells responsive to a zsig25 polypeptide, culturing a first portion of the cells in the presence of zsig25 polypeptide, culturing a second portion of the cells in the presence of the zsig25 polypeptide and a test compound, and detecting a decrease in a cellular response of the second portion of the cells as compared to the first portion of the cells. Useful antagonists of zsig25 polypeptides can also include antibodies directed against a zsig25 polypeptide epitope.

Within another aspect, samples can be tested for inhibition of zsig25 activity within a variety of assays designed to measure receptor binding or the stimulation/inhibition of zsig25-dependent cellular responses. For example, zsig25-responsive cell lines can be transfected with a reporter gene construct that is responsive to a zsig25-stimulated cellular pathway. Reporter gene constructs of this type are known in the art, and will generally comprise a zsig25-DNA response element operably linked to a gene encoding an assayable

protein, such as luciferase. DNA response elements can include, but are not limited to, cyclic AMP response elements (CRE), hormone response elements (HRE) insulin response element (IRE) (Nasrin et al., Proc. Natl. Acad. Sci. USA 87:5273-7, 1990) and serum response elements (SRE) (Shaw et al. Cell 56: 563-72, 1989). Cyclic AMP response elements are reviewed in Roestler et al., J. Biol. Chem. 263 (19):9063-6; 1988 and Habener, Molec. Endocrinol. 4 (8):1087-94; 1990. Hormone response elements are reviewed in Beato, Cell 56:335-44; 1989. Candidate compounds, solutions, mixtures or extracts are tested for the ability to inhibit the activity of zsig25 on the target cells as evidenced by a decrease in zsig25 stimulation of reporter gene expression. Assays of this type will detect compounds that directly block zsig25 binding to cell-surface receptors, as well as compounds that block processes in the cellular pathway subsequent to receptor-ligand binding. In the alternative, compounds or other samples can be tested for direct blocking of zsig25 binding to receptor using zsig25 tagged with a detectable label (e.g., ^{125}I, biotin, horseradish peroxidase, FITC, and the like). Within assays of this type, the ability of a test sample to inhibit the binding of labeled zsig25 to the receptor is indicative of inhibitory activity, which can be confirmed through secondary assays. Receptors used within binding assays may be cellular receptors or isolated, immobilized receptors.

Antibodies directed to zsig25 polypeptides of the present invention or anti-idiotypic antibodies thereof may be employed in therapeutic applications of the present invention. For example, if injected radiolabeled adenocarcinoma cells circulate or transmigrate into tissues when studied in an in vivo animal model, neutralizing anti-idiotypic antibodies with respect to such circulation (e.g., antibodies disrupting tumor cell-platelet binding) or transmigration (e.g., antibodies

disrupting tumor cell-epithelial cell or tumor cell-extracellular matrix binding) may be useful as a pretreatment to prevent metastases.

In addition, thrombospondin is known to inhibit angiogenesis, a necessary step in tumor growth and metastasis. Zsig25 polypeptides may also modulate angiogenesis. Moieties that inhibit angiogenesis may be useful in the treatment of solid tumors. Angiogenesis involves re-entry of vascular endothelial cells into the cell cycle, degradation of underlying basement membrane, and migration to form new capillary sprouts. These cells then differentiate, and mature vessels are formed. This process of growth and differentiation is regulated by a balance pro-angiogenic and anti-angiogenic factors. Mitogenic activity can be measured using known assays, including ^{3}H-thymidine incorporation assays (as disclosed by, e.g., Raines and Ross, Methods Enzymol. 109:749-73, 1985) or cell counts.

A preferred mitogenesis assay measures the incorporation of [^{3}H]-thymidine into vascular smooth muscle cells or fibroblasts. Within a typical such assay, human dermal fibroblasts are plated at a density of approximately 8,000 cells/well in 24-well culture plates and grown for approximately 72 hours in a suitable culture medium, such as DMEM containing 10% fetal calf serum. The cells are allowed to become quiescent, then exposed to a test solution. After a period of time, typically about 24 hours, [^{3}H]-thymidine is added and incubation is continued to allow growing cells to incorporate the label. The cells are then harvested, and incorporation of label is determined according to standard procedures. See also, Gospodarowicz et al., J. Cell. Biol. 70:395-405, 1976; Ewton and Florini, Endocrinol. 106:577-83, 1980; and Gospodarowicz et al., Proc. Natl. Acad. Sci. USA 86:7311-5, 1989. Thus, angiogenesis-modulating function of zsig25

polypeptides, agonists and antagonists thereof can be determined using assays known to those skilled in the art.

Moreover, the function of the prostate gland is to produce the fluid component of the ejaculate. Dysfunction of the prostate gland may result in, for example, more highly viscous ejaculate which may impede the fertilization process. Infertility caused in this manner could be impacted by adhesion-modulating polypeptides expressed by or otherwise found in prostate tissue. Such polypeptides, including the zsig25 polypeptides of the present invention, may be useful as infertility markers. In addition, inhibitors or antagonists thereof would be useful to treat the dysfunction.

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar sized regions of SEQ ID NO.1, SEQ ID NO.3, **SEQ ID NO.25,** SEQ ID NO. 5 (an oligonucleotide primer designated ZC12352), SEQ ID NO. 6 (an oligonucleotide primer designated ZC12490), SEQ ID NO. 7 (an oligonucleotide primer designated ZC12491), SEQ ID NO. 8 (an oligonucleotide primer designated ZC13387), SEQ ID NO. 9 (an oligonucleotide primer designated ZC13388), SEQ ID NO. 10 (an oligonucleotide primer designated ZC12389), SEQ ID NO. 11 (an oligonucleotide primer designated ZC13394), SEQ ID NO. 12 (an oligonucleotide primer designated ZC13455), SEQ ID NO. 13 (an oligonucleotide primer designated ZC12456), SEQ ID NO. 14 (an oligonucleotide primer designated ZC13457), SEQ ID NO. 15 (an oligonucleotide primer designated ZC13990), other probes recited herein or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence

hybridizes to a perfectly matched probe. Typical stringent conditions are those in which the salt concentration is less than about **0.03 M** at pH 7 and the temperature is at least about 60°C.

As previously noted, the isolated zsig25 polynucleotides of the present invention include DNA and RNA. Methods for isolating DNA and RNA are well known in the art. It is generally preferred to isolate RNA from prostate, although DNA can also be prepared using RNA from other tissues or isolated as genomic DNA. Total RNA can be prepared using guanidine HCl extraction followed by isolation by centrifugation in a CsCl gradient (Chirgwin et al., Biochemistry 18:52-94, 1979). Poly $(A)^+$ RNA is prepared from total RNA using the method of Aviv and Leder (Proc. Natl. Acad. Sci. USA 69:1408-12, 1972). Complementary DNA (cDNA) is prepared from poly$(A)^+$ RNA using known methods. Polynucleotides encoding zsig25 polypeptides are then identified and isolated by, for example, hybridization or PCR.

The present invention further provides counterpart polypeptides and polynucleotides from other species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of particular interest are zsig25 orthologs from other mammalian species, including murine, porcine, ovine, bovine, canine, feline, equine and other primate proteins. Orthologs of the human proteins can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses the protein. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue of cell line. A zsig25

polypeptide-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent 4,683,202), using primers designed from the sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to zsig25. Similar techniques can also be applied to the isolation of genomic clones.

ESTs encoding a murine zsig25 ortholog have been identified. These include EST 917071 (SEQ ID NO:38), EST 1274796 (SEQ ID NO:39), EST 1313542 (SEQ ID NO:40) and EST 707553 (SEQ ID NO:41) which correspond to the region of zsig25 between nucleotide 189 and 400 of SEQ ID NO:1 and EST 1377538 (SEQ ID NO:42) corresponding to the region of zsig25 between nucleotides 688 and 1055 of SEQ ID NO:1. There is a 56% identity between the mouse sequences and human zsig25.

Alternate species polypeptides of zsig25 may have importance therapeutically. It has been demonstrated that in some cases use of a non-native protein, i.e., protein from a different species, can be more potent than the native protein. For example, salmon calcitonin has been shown to be considerably more effective in arresting bone resorption than human forms of calcitonin. There are several hypotheses as to why salmon calcitonin is more potent than human calcitonin in treatment of osteoporosis. These hypotheses include: 1) salmon calcitonin is more resistant to degradation; 2) salmon calcitonin has a lower metabolic clearance rate (MCR); and 3) salmon calcitonin may have a slightly different conformation, resulting in a higher affinity for bone receptor sites. Another example is found in the β-endorphin family (Ho et

al., Int. J. Peptide Protein Res. 29:521-24, 1987). Studies have demonstrated that the peripheral opioid activity of camel, horse, turkey and ostrich β-endorphins is greater than that of human β-endorphins when isolated guinea pig ileum was electrostimulated and contractions were measured. Vas deferens from rat, mouse and rabbit were assayed as well. In the rat vas deferens model, camel and horse β-endorphins showed the highest relative potency. Synthesized rat relaxin was as active as human and porcine relaxin in the mouse symphysis pubis assay (Bullesbach and Schwabe, Eur. J. Biochem. 241:533-7, 1996). Thus, the mouse zsig25 molecules of the present invention may have higher potency than the human endogenous molecule in human cells, tissues and recipients.

Those skilled in the art will recognize that the sequences disclosed in SEQ ID NO.1 and SEQ ID NO.2 represent a single allele of the human zsig25 gene and polypeptide, and that allelic variation and alternative splicing are expected to occur. For example, Northern blot analysis revealed 2 kb and 5 kb transcripts, wherein the 2 kb transcript was more highly expressed. In fetal blots, a 2.2 kd and 6.0 kd transcript were detected. Such transcripts may represent splice variants. In addition, allelic variants can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the DNA sequence shown in SEQ ID NO.1, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of SEQ ID NO:2. cDNAs generated from alternatively spliced mRNAs, which retain the properties of the zsig25 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be

cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

The present invention also provides isolated zsig25 polypeptides that are substantially homologous to the polypeptides of SEQ ID NO.2 and their species homologs/orthologs. The term "substantially homologous" is used herein to denote polypeptides having 50%, preferably 60%, more preferably at least 80%, sequence identity to the sequences shown in SEQ ID NO.2 or their orthologs. Such polypeptides will more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO.2 or its orthologs. Percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48: 603-16, 1986 and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-9, 1992. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blosum 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown in Table 3 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{\left[\begin{array}{l}\text{length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences}\end{array}\right]} \times 100$$

Table 3

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

Substantially homologous proteins and polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 4) and other substitutions that do not significantly affect the folding or activity of the protein or polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification (an affinity tag), such as a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al., Methods Enzymol. 198:3, 1991), glutathione S transferase (Smith and Johnson, Gene 67:31, 1988), Glu-Glu, maltose binding protein (Kellerman and Ferenci, Methods Enzymol. 90:459-463, 1982; Guan et al., Gene 67:21-30, 1987), thioredoxin, ubiquitin, cellulose binding protein, T7 polymerase, or other antigenic epitope or binding domain. See, in general Ford et al., Protein Expression and Purification 2: 95-107, 1991. DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ; New England Biolabs, Beverly, MA). Polypeptides comprising affinity tags can further comprise a proteolytic cleavage site between the zsig25 polypeptide and the affinity tag. Preferred such sites include thrombin cleavage sites and factor Xa cleavage sites.

Table 4

Conservative amino acid substitutions

Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Polar:	glutamine
	asparagine
Hydrophobic:	leucine
	isoleucine
	valine
Aromatic:	phenylalanine
	tryptophan
	tyrosine
Small:	glycine
	alanine
	serine
	threonine
	methionine

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed

using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722, 1991; Ellman et al., Methods Enzymol. 202:301, 1991; Chung et al., Science 259:806-9, 1993; and Chung et al., Proc. Natl. Acad. Sci. USA 90:10145-9, 1993). In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991-8, 1996). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide et al., Biochem. 33:7470-6, 1994. Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395-403, 1993).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for zsig25 amino acid residues.

Hydrophilicity can be determined, as shown in Figure 8. Hydrophilicity can be used to determine regions that have the most antigenic potential. For example, in

zsig25, hydrophilic regions include amino acid residues 174-179 of SEQ ID NO: 2, amino acid residues 172-177 of SEQ ID NO: 2, amino acid residues 320-325 of SEQ ID NO: 2, amino acid residues 317-322 of SEQ ID NO: 2, and amino acid residues 141-146 of SEQ ID NO: 2.

Essential amino acids in the zsig25 polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244: 1081-5, 1989). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (e.g., adhesion-modulation, differentiation-modulation or the like) to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., J. Biol. Chem. 271:4699-708, 1996. Sites of ligand-receptor or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., Science 255:306-12, 1992; Smith et al., J. Mol. Biol. 224:899-904, 1992; Wlodaver et al., FEBS Lett. 309:59-64, 1992. The identities of essential amino acids can also be inferred from analysis of homologies with related proteins, such as rat F-spondin protein.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53-57, 1988) or Bowie and Sauer (Proc. Natl. Acad. Sci. USA 86:2152-2156, 1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the

mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., Biochem. 30:10832-10837, 1991; Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis (Derbyshire et al., Gene 46:145, 1986; Ner et al., DNA 7:127, 1988).

Variants of the disclosed zsig25 DNA and polypeptide sequences can be generated through DNA shuffling as disclosed by Stemmer, Nature 370:389-91, 1994, Stemmer, Proc. Natl. Acad. Sci. USA 91:10747-51, 1994 and WIPO Publication WO 97/20078. Briefly, variant DNAs are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNAs, such as allelic variants or DNAs from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed above can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode active polypeptides (e.g., prostate cell proliferation-, adhesion- or signaling-modulation, B-cell proliferation or the like) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed above, one of ordinary skill in the art can identify and/or prepare a

variety of polypeptides that are substantially homologous to residues 27 to 331 of SEQ ID NO. 2 or allelic variants thereof and retain the adhesion-modulating, differentiation-modulating, cellular proliferating or like properties of the wild-type protein. Such polypeptides may include additional amino acids, such as affinity tags and the like. Such polypeptides may also include additional polypeptide segments as generally disclosed herein.

The polypeptides of the present invention, including full-length proteins, fragments thereof and fusion proteins, can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, and Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987.

In general, a DNA sequence encoding a zsig25 polypeptide of the present invention is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and

other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a zsig25 polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of the zsig25 polypeptide, or may be derived from another secreted protein (e.g., t-PA) or synthesized *de novo*. The secretory signal sequence is joined to the zsig25 DNA sequence in the correct reading frame and positioned to direct newly synthesized polypeptide into secretory pathways to host cell. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830). Conversely, the secretory signal sequence portion of the zsig25 polypeptide (amino acids 1-26 of SEQ ID NO. 2) may be employed to direct the secretion of an alternative protein by analogous methods.

Alternatively, the secretory signal sequence contained in the polypeptides of the present invention is used to direct other polypeptides into the secretory pathway. The present invention provides for such fusion polypeptides. A signal fusion polypeptide can be made wherein a secretory signal sequence derived from amino acid residues 1-26 of SEQ ID NO:2 is be operably linked to another polypeptide using methods known in the art and disclosed herein. The secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory

pathway. Such constructs have numerous applications known in the art. For example, these novel secretory signal sequence fusion constructs can direct the secretion of an active component of a normally non-secreted protein, such as a receptor. Such fusions may be used *in vivo* or *in vitro* to direct peptides through the secretory pathway.

Cultured mammalian cells are suitable hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler et al., Cell 14:725, 1978; Corsaro and Pearson, Somatic Cell Genetics 7:603, 1981: Graham and Van der Eb, Virology 52:456, 1973), electroporation (Neumann et al., EMBO J. 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987), liposome-mediated transfection (Hawley-Nelson et al., Focus 15:73, 1993; Ciccarone et al., Focus 15:80, 1993), and viral vectors (Miller and Rosman, BioTechniques 7:980-90, 1989; Wang and Finer, Nature Med. 2:714-16, 1996). The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., J. Gen. Virol. 36:59-72, 1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos.

4,579,821 and 4,601,978) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g., hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternative markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

Other higher eukaryotic cells can also be used as hosts, including plant cells, insect cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al., J. Biosci. (Bangalore) 11:47-58, 1987. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino et al., U.S.

Patent No. 5,162,222 and WIPO publication WO 94/06463. Insect cells can be infected with recombinant baculovirus, commonly derived from Autographa californica nuclear polyhedrosis virus (AcNPV). DNA encoding the zsig25 polypeptide is inserted into the baculoviral genome in place of the AcNPV polyhedrin gene coding sequence by one of two methods. The first is the traditional method of homologous DNA recombination between wild-type AcNPV and a transfer vector containing the zsig25 flanked by AcNPV sequences. Suitable insect cells, e.g. SF9 cells, are infected with wild-type AcNPV and transfected with a transfer vector comprising a zsig25 polynucleotide operably linked to an AcNPV polyhedrin gene promoter, terminator, and flanking sequences. See, King and Possee, The Baculovirus Expression System: A Laboratory Guide, London, Chapman & Hall; O'Reilly et al., Baculovirus Expression Vectors: A Laboratory Manual, New York, Oxford University Press., 1994; and, Richardson, C. D., Ed., Baculovirus Expression Protocols. Methods in Molecular Biology, Totowa, NJ, Humana Press, 1995. Natural recombination within an insect cell will result in a recombinant baculovirus which contains zsig25 driven by the polyhedrin promoter. Recombinant viral stocks are made by methods commonly used in the art.

The second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow et al., J. Virol. 67:4566-79, 1993). This system is sold in the Bac-to-Bac kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a Tn7 transposon to move the DNA encoding the zsig25 polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." The pFastBac1™ transfer vector utilizes the AcNPV polyhedrin promoter to drive the expression of the gene of interest, in this case zsig25. However, pFastBac1™ can be modified to a

considerable degree. The polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as P*cor*, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins. See, Hill-Perkins and Possee, J. Gen. Virol. 71:971-6, 1990; Bonning et al., J. Gen. Virol. 75:1551-6, 1994; and, Chazenbalk, G.D., and Rapoport, J. Biol. Chem. 270:1543-9, 1995. In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed which replace the native zsig25 secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen, Carlsbad, CA), or baculovirus gp67 (PharMingen, San Diego, CA) can be used in constructs to replace the native zsig25 secretory signal sequence. In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed zsig25 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer et al., ibid.). Using a technique known in the art, a transfer vector containing zsig25 is transformed into *E. coli*, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, e.g. Sf9 cells. Recombinant virus that expresses zsig25 is subsequently produced. Recombinant viral stocks are made by methods commonly used the art.

The recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *Spodoptera frugiperda*. See, in general, Glick and Pasternak, Molecular Biotechnology: Principles and Applications of Recombinant DNA, ASM Press, Washington,

D.C., 1994. Another suitable cell line is the High Five™ cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent #5,300,435). Commercially available serum-free media are used to grow and maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cell0405™ (JRH Biosciences, Lenexa, KS) or Express Five™ (Life Technologies) for the *T. ni* cells. The cells are grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. The recombinant virus-infected cells typically produce the recombinant zsig25 polypeptide at 12-72 hours post-infection and secrete it with varying efficiency into the medium. The culture is usually harvested 48 hours post-infection. Centrifugation is used to separate the cells from the medium (supernatant). The supernatant containing the zsig25 polypeptide is filtered through micropore filters, usually 0.45 μm pore size. Procedures used are generally described in available laboratory manuals (King and Possee, ibid.; O'Reilly et al., ibid.; Richardson, C. D., ibid.). Subsequent purification of the zsig25 polypeptide from the supernatant can be achieved using methods described herein.

Fungal cells, including yeast cells, can also be used within the present invention. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845,075. Transformed cells are selected by

phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki et al. (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., J. Gen. Microbiol. 132:3459-65, 1986 and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

The use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed in WIPO Publications WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, it is preferred that the promoter and terminator in the plasmid be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene

(*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A preferred selectable marker for use in *Pichia methanolica* is a *P. methanolica ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, it is preferred to use host cells in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells deficient in vacuolar protease genes (*PEP4* and *PRB1*) are preferred. Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. It is preferred to transform *P. methanolica* cells by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (τ) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art (see, e.g., Sambrook et al., ibid.). When expressing a zsig25 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate

or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell. *P. methanolica* cells are cultured in a medium comprising adequate sources of carbon, nitrogen and trace nutrients at a temperature of about 25°C to 35°C. Liquid cultures are provided with sufficient aeration by conventional means, such as shaking of small flasks or sparging of fermentors. A preferred culture medium for *P. methanolica* is YEPD (2% D-glucose, 2% Bacto™ Peptone (Difco Laboratories, Detroit, MI), 1% Bacto™ yeast extract (Difco Laboratories), 0.004% adenine and 0.006% L-leucine).

Proteins of the present invention are useful for adhesion modulation, for example. Such activity can be

measured *in vitro* using cultured cells or *in vivo* by administering molecules of the claimed invention to the appropriate animal model. For instance, zsig25 transfected (or co-transfected) expression host cells may be embedded in an alginate environment and injected (implanted) into recipient animals. Alginate-poly-L-lysine microencapsulation, permselective membrane encapsulation and diffusion chambers have been described as a means to entrap transfected mammalian cells or primary mammalian cells. These types of non-immunogenic "encapsulations" or microenvironments permit the transfer of nutrients into the microenvironment, and also permit the diffusion of proteins and other macromolecules secreted or released by the captured cells across the environmental barrier to the recipient animal. Most importantly, the capsules or microenvironments mask and shield the foreign, embedded cells from the recipient animal's immune response. Such microenvironments can extend the life of the injected cells from a few hours or days (naked cells) to several weeks (embedded cells).

Alginate threads provide a simple and quick means for generating embedded cells. The materials needed to generate the alginate threads are readily available and relatively inexpensive. Once made, the alginate threads are relatively strong and durable, both *in vitro* and, based on data obtained using the threads, *in vivo*. The alginate threads are easily manipulable and the methodology is scalable for preparation of numerous threads. In an exemplary procedure, 3% alginate is prepared in sterile H_2O, and sterile filtered. Just prior to preparation of alginate threads, the alginate solution is again filtered. An approximately 50% cell suspension (containing about 5 x 10^5 to about 5 x 10^7 cells/ml) is mixed with the 3% alginate solution. One ml of the alginate/cell suspension is extruded into a 100 mM sterile filtered $CaCl_2$ solution over a time period of ~15 min,

forming a "thread". The extruded thread is then transferred into a solution of 50 mM $CaCl_2$, and then into a solution of 25 mM $CaCl_2$. The thread is then rinsed with deionized water before coating the thread by incubating in a 0.01% solution of poly-L-lysine. Finally, the thread is rinsed with Lactated Ringer's Solution and drawn from solution into a syringe barrel (without needle attached). A large bore needle is then attached to the syringe, and the thread is intraperitoneally injected into a recipient in a minimal volume of the Lactated Ringer's Solution.

An alternative *in vivo* approach for assaying proteins of the present invention involves viral delivery systems. Exemplary viruses for this purpose include adenovirus, herpesvirus, vaccinia virus and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for a review, see T.C. Becker et al., Meth. Cell Biol. 43:161-89, 1994; and J.T. Douglas and D.T. Curiel, Science & Medicine 4:44-53, 1997). The adenovirus system offers several advantages: adenovirus can (i) accommodate relatively large DNA inserts; (ii) be grown to high-titer; (iii) infect a broad range of mammalian cell types; and (iv) be used with a large number of available vectors containing different promoters. Also, because adenoviruses are stable in the bloodstream, they can be administered by intravenous injection. Some disadvantages (especially for gene therapy) associated with adenovirus gene delivery include: (i) very low efficiency integration into the host genome; (ii) existence in primarily episomal form; and (iii) the host immune response to the administered virus, precluding readministration of the adenoviral vector.

By deleting portions of the adenovirus genome, larger inserts (up to 7 kb) of heterologous DNA can be accommodated. These inserts can be incorporated into the viral DNA by direct ligation or by homologous

recombination with a co-transfected plasmid. In an exemplary system, the essential E1 gene has been deleted from the viral vector, and the virus will not replicate unless the E1 gene is provided by the host cell (the human 293 cell line is exemplary). When intravenously administered to intact animals, adenovirus primarily targets the liver. If the adenoviral delivery system has an E1 gene deletion, the virus cannot replicate in the host cells. However, the host's tissue (e.g., liver) will express and process (and, if a secretory signal sequence is present, secrete) the heterologous protein. Secreted proteins will enter the circulation in the highly vascularized liver, and effects on the infected animal can be determined.

The adenovirus system can also be used for protein production *in vitro*. By culturing adenovirus-infected non-293 cells under conditions where the cells are not rapidly dividing, the cells can produce proteins for extended periods of time. For instance, BHK cells are grown to confluence in cell factories, then exposed to the adenoviral vector encoding the secreted protein of interest. The cells are then grown under serum-free conditions, which allows infected cells to survive for several weeks without significant cell division. Alternatively, adenovirus vector infected 293S cells can be grown in suspension culture at relatively high cell density to produce significant amounts of protein (see Garnier et al., Cytotechnol. 15:145-55, 1994). With either protocol, an expressed, secreted heterologous protein can be repeatedly isolated from the cell culture supernatant. Within the infected 293S cell production protocol, non-secreted proteins may also be effectively obtained.

Expressed recombinant zsig25 polypeptides (or chimeric zsig25 polypeptides) can be purified using fractionation and/or conventional purification methods and

media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable anion exchange media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. DEAE Fast-Flow Sepharose (Pharmacia, Piscataway, NJ), PEI, DEAE, QAE and Q derivatives are preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, Affinity Chromatography: Principles & Methods, Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988.

The zsig25 polypeptides of the present invention can be isolated by exploitation of their structural features. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, Trends in Biochem. 3:1-7, 1985). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (Methods in Enzymol., Vol. 182, "Guide to Protein Purification", M. Deutscher, (ed.), Acad. Press, San Diego, 1990, pp.529-39). Within additional embodiments of the invention, a fusion of the polypeptide of interest and an affinity tag (e.g., polyhistidine, Glu-Glu, FLAG, maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

Isolated and purified human zsig25 polypeptide fragments are encompassed by the present invention. Preferably fragments (1) do not appreciably bind to or otherwise associate with extracellular matrix or epithelial cells. (2) bind or otherwise associate with prostate cells or prostate cancer cells. Preferably, such fragments upregulate cell-cell adhesion of prostate or prostate cancer cells. The binding ability of the zsig25 polypeptide fragments with regard to extracellular matrix components, epithelial cells, prostate cells and prostate cancer cells can be assessed by binding assays known to those skilled in the art. Also, the adhesion function of the zsig25 polypeptide fragment can be assessed as described above. These polypeptide fragments are useful for studying cell adhesion and the role thereof in

metastasis and may be useful in preventing metastasis, in particular metastasis of prostate tumors. 3) stimulate cellular proliferation and differentiation. Assays to measure cellular proliferation and differentiation are known within the art and representative assays are disclosed herein.

Also, isolated and purified fusion proteins formed from (1) human zsig25 polypeptide fragments discussed above or human zsig25 protein fragments encompassing an amino acid sequence as shown in SEQ ID NO. 2 from amino acid residue 27 (Gln) to amino acid residue 277 (Cys) or orthologs thereof and (2) a C-terminal region incorporating from about 2 to about 8 thrombospondin type 1 repeats, such as the C-terminal region of rat F-spondin, human thrombospondin I, human thrombospondin II or the like. Fusion proteins can be prepared by methods known to those skilled in the art by preparing each component of the fusion protein and chemically conjugating them. Preferably the thrombospondin type 1 repeats are fused carboxy-terminally to the zsig25 polypeptide. Alternatively, a polyoligonucleotide encoding both components of the fusion protein in the proper reading frame may be generated using known techniques and expressed by the methods described herein. Such fusion proteins would be expected to have a biological functional profile that is the same or similar to zsig25 polypeptides of the present invention, although such fusion proteins may exhibit more pronounced adhesion-modulating properties.

Auxiliary domains can be fused to zsig25 polypeptides to target them to specific cells, tissues, or macromolecules (e.g., prostate). For example, a zsig25 polypeptide or protein could be targeted to a predetermined cell type by fusing a zsig25 polypeptide to a ligand that specifically binds to a receptor on the surface of the target cell. In this way, polypeptides and

proteins can be targeted for therapeutic or diagnostic purposes. A zsig25 polypeptide can be fused to two or more moieties, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, Tuan et al., Connective Tissue Research 34:1-9, 1996.

Protein refolding (and optionally reoxidation) procedures may be advantageously used. It is preferred to purify the protein to >80% purity, more preferably to >90% purity, even more preferably >95%, and particularly preferred is a pharmaceutically pure state, that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. Preferably, a purified protein is substantially free of other proteins, particularly other proteins of animal origin.

Zsig25 polypeptides or fragments thereof may also be prepared through chemical synthesis. Zsig25 polypeptides may be monomers or multimers; glycosylated or non-glycosylated; pegylated or non-pegylated; and may or may not include an initial methionine amino acid residue.

A zsig25 ligand-binding polypeptide can also be used for purification of ligand. The ligand-binding polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting medium will generally be configured in the form of a column, and fluids containing zsig25 polypeptide are

passed through the column one or more times to allow zsig25 polypeptide to bind to the ligand-binding or receptor polypeptide. The bound zsig25 polypeptide is then eluted using changes in salt concentration, chaotropic agents (guanidine HCl), or pH to disrupt ligand-receptor binding.

An assay system that uses a ligand-binding receptor (or an antibody, one member of a complement/anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument (BIAcore™, Pharmacia Biosensor, Piscataway, NJ) may be advantageously employed. Such receptor, antibody, member of a complement/anti-complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, J. Immunol. Methods 145:229-40, 1991 and Cunningham and Wells, J. Mol. Biol. 234:554-63, 1993. A receptor, antibody, member or fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If a ligand, epitope, or opposite member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

Zsig25 polypeptide and other ligand homologs can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity (see Scatchard, Ann. NY Acad. Sci. 51: 660-72, 1949) and calorimetric assays (Cunningham et al., Science 253:545-48, 1991; Cunningham et al., Science 245:821-25, 1991).

Zsig25 polypeptides can also be used to prepare antibodies that specifically bind to zsig25 epitopes, peptides or polypeptides. Methods for preparing polyclonal and monoclonal antibodies are well known in the art (see, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boca Raton, FL, 1982). As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from a variety of warm-blooded animals, such as horses, cows, goats, sheep, dogs, chickens, hamsters, guinea pigs, rabbits, mice, and rats, as well as transgenic animals such as transgenic sheep, cows, goats or pigs. Antibodies may also be expressed in yeast and fungi in modified forms as well as in mammalian and insect cells.

The zsig25 polypeptide or a fragment thereof serves as an antigen (immunogen) to inoculate an animal or elicit an immune response. Suitable antigens would include the zsig25 polypeptide encoded by SEQ ID NO:2 from amino acid residue 27-331 of SEQ ID NO:2, or a contiguous 9-331 amino acid residue fragment thereof. The immunogenicity of a zsig25 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of zsig25 polypeptide or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as $F(ab')_2$ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced. Human antibodies can also be made in mice having a humanized humoral immune system (Mendez et al., Nat. Genet. 14:146- 56, 1997). Alternative techniques for generating or selecting antibodies useful herein include *in vitro* exposure of lymphocytes to zsig25 protein or peptide, and selection of antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled zsig25 protein or peptide). Mutagenesis methods discussed herein, in particular domain shuffling, can be used to generate and mature antibodies.

The antibodies of the current invention, or fragments thereof, can be used to direct molecules to a specific target. For example, as T-bodies, chimeric receptors combining antibody recognition with T cell effector function, (Eshhar et al., Springer Semin Immunopathol. 18:199-209, 1996; Eshhar, Cancer Immunol. Immunother. 45:131-6, 1997). Intrabodies, engineered

single-chain antibodies expressed inside the cell and having high affinity and specificity for intracellular targets. Such molecules have use in gene therapy and treatment of infectious diseases (Marasco, Immunotechnology 1:1-19, 1995; Marasco et al., Gene Ther. 4:11-5, 1997; Rondon and Marasco, Annu. Rev. Microbiol. 51:257-83, 1997 and Mhashilkar et al., J. Virol. 71:6486-94, 1997). Diabodies, bispecific non-covalent dimers of scFv antibodies useful for immunodiagnosis and therapeutically. In addition they can be constructed in bacteria (Holliger et al., Proc. Natl. Acad. Sci. USA 90:6444-48, 1993).

Antibodies are defined to be specifically binding if they bind to a zsig25 polypeptide with a binding affinity (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably 10^9 M^{-1} or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art (for example, by Scatchard analysis).

Genes encoding polypeptides having potential zsig25 polypeptide binding domains, "binding proteins", can be obtained by screening random or directed peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. Alternatively, constrained phage display libraries can also be produced. These peptide display libraries can be used to screen for peptides which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such peptide display libraries are known in the art (Ladner et al., US Patent NO. 5,223,409; Ladner et al., US Patent NO. 4,946,778; Ladner et al., US Patent NO. 5,403,484 and

Ladner et al., US Patent NO. 5,571,698) and peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Peptide display libraries can be screened using the zsig25 sequences disclosed herein to identify proteins which bind to zsig25. These "binding proteins" which interact with zsig25 polypeptides can be used essentially like an antibody, for tagging cells; for isolating homolog polypeptides by affinity purification; directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding proteins can also be used in analytical methods such as for screening expression libraries and neutralizing activity. The binding proteins can also be used for diagnostic assays for determining circulating levels of polypeptides; for detecting or quantitating soluble polypeptides as marker of underlying pathology or disease. To increase the half-life of these binding proteins, they can be conjugated. Their biological properties may be modified by dimerizing or multimerizing for use as agonists or antagonists.

A variety of assays known to those skilled in the art can be utilized to detect antibodies and binding proteins which specifically bind to zsig25 proteins or peptides. Exemplary assays are described in detail in Antibodies: A Laboratory Manual, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmuno-precipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can be screened for binding to wild-type versus mutant zsig25 protein or peptide.

Antibodies to zsig25 may be used for tagging cells that express zsig25 polypeptide; for isolating zsig25 polypeptide by affinity purification; for diagnostic assays for determining circulating levels of zsig25 polypeptides; for detecting or quantitating soluble zsig25 polypeptide as marker of underlying pathology or disease; in analytical methods employing FACS; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block zsig25 cell adhesion activity *in vitro* and *in vivo*. Suitable direct tags or labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. Antibodies herein may also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications.

Molecules of the present invention can be used to identify and isolate receptors involved in prostate cell adhesion, motility, proliferation, cell signaling or the like. For example, proteins and peptides of the present invention can be immobilized on a column and membrane preparations run over the column (Immobilized Affinity Ligand Techniques, Hermanson et al., eds., Academic Press, San Diego, CA, 1992, pp.195-202). Proteins and peptides can also be radiolabeled (Methods in Enzymol., vol. 182, "Guide to Protein Purification", M. Deutscher, ed., Acad. Press, San Diego, 1990, 721-737) or photoaffinity labeled (Brunner et al., Ann. Rev. Biochem. 62:483-514, 1993 and Fedan et al., Biochem. Pharmacol. 33:1167-1180, 1984) and specific cell-surface proteins can be identified.

For pharmaceutical use, the proteins of the present invention may be formulated for parenteral

delivery, preferably intravenous or subcutaneous delivery, according to conventional methods. Intravenous administration of therapeutic antibodies, small molecule antagonists or agonists of zsig25 polypeptides, or zsig25 polypeptide fragments of the present invention will preferably be by bolus injection or infusion over a typical period of one to several hours. Intravenous administration of zsig25 polypeptides may be a sub-optimal route of administration to deliver the polypeptide to prostate or other solid tissue. In such circumstances, direct injection into the afflicted site or other routes of administration with preference for such afflicted sites (e.g., hepatic artery administration to liver sites) may be employed. Choice of an appropriate route of administration is within the ordinary skill in the art.

In general, pharmaceutical formulations will include a therapeutic component in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc. As used herein, a pharmaceutically effective amount of a zsig25 polypeptide, agonist or antagonist, is an amount sufficient to induce a desired biological result. The result can be alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. For example, an effective amount of a polypeptide of the present invention is that which provides either subjective relief of symptoms or an objectively identifiable improvement as noted by the clinician or other qualified observer. In particular, such an effective amount results in reduction or alleviation of an inflammatory response in the liver, proliferation of B-cells, delay in platelet formation or other beneficial effect. Methods of formulation are well

known in the art and are disclosed, for example, in Remington: The Science and Practice of Pharmacy, Gennaro, ed., Mack Publishing Co., Easton, PA, 19th ed., 1995. Therapeutic doses of antagonists will depend upon the percentage of antagonism desired. Doses sufficient to produce between 20% to 100% antagonism may be used in the practice of the present invention, with greater than 70% antagonism preferred and greater than 90% antagonism more preferred. Doses of zsig25 polypeptide will generally be determined by the clinician according to accepted standards, taking into account the nature and severity of the condition to be treated, patient traits, etc. Determination of dose is within the level of ordinary skill in the art. The proteins may be administered for acute treatment, over one week or less, often over a period of one to three days or may be used in chronic treatment, over several months or years.

Polynucleotides encoding zsig25 polypeptides are useful within gene therapy applications where it is desired to increase or inhibit zsig25 activity. If a mammal has a mutated or absent zsig25 gene, the zsig25 gene can be introduced into the cells of the mammal. In one embodiment, a gene encoding a zsig25 polypeptide is introduced *in vivo* in a viral vector. Such vectors include an attenuated or defective DNA virus, such as, but not limited to, herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. A defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Examples of particular vectors include, but are not limited to, a defective herpes simplex virus 1 (HSV1) vector (Kaplitt et al., Molec. Cell. Neurosci.

2:320-30, 1991); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al., J. Clin. Invest. 90:626-30, 1992; and a defective adeno-associated virus vector (Samulski et al., J. Virol. 61:3096-101, 1987; Samulski et al., J. Virol. 63:3822-8, 1989). Such gene therapy methods would be useful for treating Wolf-Hirschhorn Syndrome.

In another embodiment, a zsig25 gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Patent No. 5,399,346; Mann et al. Cell 33:153, 1983; Temin et al., U.S. Patent No. 4,650,764; Temin et al., U.S. Patent No. 4,980,289; Markowitz et al., J. Virol. 62:1120, 1988; Temin et al., U.S. Patent No. 5,124,263; International Patent Publication No. WO 95/07358, published March 16, 1995 by Dougherty et al.; and Kuo et al., Blood 82:845, 1993. Alternatively, the vector can be introduced by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7, 1987; Mackey et al., Proc. Natl. Acad. Sci. USA 85:8027-31, 1988). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. More particularly, directing transfection to particular cells represents one area of benefit. For instance, directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (e.g., hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

It is possible to remove the target cells from the body; to introduce the vector as a naked DNA plasmid; and then to re-implant the transformed cells into the body. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter. See, e.g., Wu et al., J. Biol. Chem. 267:963-7, 1992; Wu et al., J. Biol. Chem. 263:14621-4, 1988.

Antisense methodology can be used to inhibit zsig25 gene translation, such as to inhibit cell proliferation *in vivo*. Polynucleotides that are complementary to a segment of a zsig25-encoding polynucleotide (e.g., a polynucleotide as set froth in SEQ ID NO:1) are designed to bind to zsig25-encoding mRNA and to inhibit translation of such mRNA. Such antisense polynucleotides are used to inhibit expression of zsig25 polypeptide-encoding genes in cell culture or in a subject.

Transgenic mice, engineered to express the zsig25 gene, and mice that exhibit a complete absence of zsig25 gene function, referred to as "knockout mice" (Snouwaert et al., Science 257:1083, 1992), may also be generated (Lowell et al., Nature 366:740-42, 1993). These mice may be employed to study the zsig25 gene and the protein encoded thereby in an *in vivo* system.

The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1

Extension of EST Sequence

The novel zsig25 polypeptide-encoding polynucleotides of the present invention were initially identified by querying an EST database for secretory signal sequences characterized by an upstream methionine start site, a hydrophobic region of approximately 13 amino acids and a cleavage site (SEQ ID NO. 5, wherein cleavage occurs between the glycine and glutamine amino acid residues) in an effort to select for secreted proteins. Contemporaneously, a human fetal heart library was examined for polypeptides in an effort to select for proteins having interesting homology.

Polypeptides corresponding to ESTs meeting those search criteria were compared to known sequences to identify secreted proteins having homology to known ligands. An EST sequence was discovered and predicted to be related to a secreted *Xenopus* F-spondin protein. See, for example, Klar et al., Cell 69: 95-110, 1992. To identify the corresponding cDNA, two clones, zsig25a and zsig25b, considered likely in combination to contain the entire coding region were used for sequencing.

Using an Invitrogen S.N.A.P.™ Miniprep kit (Invitrogen, Corp., San Diego, CA) according to manufacturer's instructions a 5 ml overnight culture in LB + 50 µg/ml ampicillin was prepared. The template was sequenced on an ABIPRISM ™ model 377 DNA sequencer (Perkin-Elmer Cetus, Norwalk, Ct.) using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Corp.) according to manufacturer's instructions. Oligonucleotides ZC447 (SEQ ID NO. 16) and ZC976 (SEQ ID NO. 17), respectively corresponding to the M13 and LacZ promoters on the clone-containing vector,

were used as sequencing primers. Oligonucleotides ZC12490 (SEQ ID NO. 6), ZC12491 (SEQ ID NO. 7), ZC13387 (SEQ ID NO. 8), ZC13388 (SEQ ID NO. 9), ZC13455 (SEQ ID NO. 12), ZC13456 (SEQ ID NO. 13) and ZC13390 (SEQ ID NO. 15) were used to extend the sequence of the zsig25a clone. Oligonucleotides ZC13387 (SEQ ID NO. 8), ZC13388 (SEQ ID NO. 9), ZC13389 (SEQ ID NO. 10), ZC13394 (SEQ ID NO. 11), ZC13455 (SEQ ID NO. 12), ZC13456 (SEQ ID NO. 13), ZC13457 (SEQ ID NO. 14) and ZC13390 (SEQ ID NO. 15) were used to extend the sequence of the zsig25b clone.

The sequences of zsig25a and zsig25b were evaluated. Zsig25a was found to be a 1354 bp cDNA including an EcoRI linker and an oligonucleotide dT priming site. Zsig25b was found to be a 1436 bp cDNA containing a polyadenylation site downstream of a putative polyadenylation signal. Zsig25a and zsig25b clones were found to overlap for 1137 bases, sharing 100% identity in the overlapping region. Zsig25a was 188 bp longer on the 5' end and contains the putative secretory signal sequence, while zsig25b was 280 bp longer on the 3' end and contained the putative polyadenylation signal. The primers and oligonucleotides set forth above were used to determine the full length sequence of zsig25 polypeptide.

Sequencing reactions were carried out in a Hybaid OmniGene Temperature Cycling System (National Labnet Co., Woodbridge, NY). SEQUENCHER™ 3.0 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI) was used for data analysis. The resulting 1607 bp sequence is disclosed in SEQ ID NO. 1. Comparison of the originally derived EST sequences with the sequence represented in SEQ ID NO. 1 showed 98.7% identity over 151 nucleotides (zsig25a) and 88.6% identity over 272 nucleotides (zsig25b). At the amino acid level shown, for example, in SEQ ID NO. 2, comparison of the expected amino acids based on the original EST sequences showed 95.2% amino acid identity over 42 amino acids (zsig25a) and

84.4% amino acid identity over the best 77 amino acid stretch (amino acids 50-127 in SEQ ID NO. 2 which corresponded to zsig25b). Most of the lack of identity arose from ambiguity in the original EST sequences.

Example 2
Tissue Distribution

Northerns were performed using Human Multiple Tissue Blots from Clontech (Palo Alto, CA). A 33 bp DNA probe (ZC12352; SEQ ID NO. 6) to the 5' end of the oligonucleotide sequence of the mature protein shown in SEQ ID NO. 1 was radioactively labeled with ^{32}P using T4 polynucleotide kinase and forward reaction buffer (GIBCO BRL, Gaithersburg, MD) according to the manufacturer's specifications. The probe was purified using a NUCTRAP push column (Stratagene Cloning Systems, La Jolla, CA). EXPRESSHYB (Clontech, Palo Alto, CA) solution was used for prehybridization and as a hybridizing solution for the Northern blots. Hybridization took place overnight at 42° C, and the blots were then washed in 2X SSC and 0.05% SDS at RT, followed by a wash in 1X SSC and 0.1% SDS at 65°C. One transcript size was observed at approximately 2 kb. Signal intensity was highest for prostate, with relatively less intense signals in placenta ovary, small intestine and peripheral blood lymphocytes. Weaker signals were present in testis, heart, adrenal gland and colon.

Additional analysis was carried out on Northern blots made with poly(A) RNA from $CD4^+$, $CD8^+$, $CD19^+$ and mixed lymphocyte reaction cells (CellPro, Bothell, WA). Total RNA was prepared using guanidine isothiocyanate (Chirgwin et al., Biochemistry 18:52-94, 1979), followed by a CsCl centrifugation step. Poly(A)+ RNA was isolated using oligo d(T) cellulose chromatography (Aviv and Leder, Proc. Natl. Acad. Sci. USA. 69:1408-12, 1972). Northern blot analysis was then performed as follows.

About 2 µg of each of the poly A+ RNAs was denatured in 2.2 M formaldehyde/phosphate buffer (50 mM Na_2HPO_4, 50 mM NaH_2PO_4, 50 mM NaOAc, 1 mM EDTA and 2.2 M formaldehyde) and separated by 1.5% agarose mini gel (Stratagene Cloning Systems, La Jolla, CA) electrophoresis in formaldehyde/phosphate buffer. The RNA was blotted overnight onto a nytran filter (Schleicher & Schuell, Keene, NH), and the filter was UV crosslinked (1,200 µJoules) in a STRATALINKER® UV crosslinker (Stratagene Cloning Systems) and then baked at 80°C for 1 hour.

The 1000 bp zsig25 Eco RI and Bam HI restriction fragment, from the mammalian expression vector zSIG25/NFpZP9 as described in below, was used as a probe. The resulting DNA fragment was electrophoresed on a 1.5% agarose gel (UltraPure agarose, GIBCO BRL, Gaithersburg, MD), the fragment was purified using the QIAEX™ method (Qiagen, Chatsworth, CA), and the sequence was confirmed by sequence analysis. The probe was radioactively labeled using the random priming MULTIPRIME DNA labeling system (Amersham, Arlington Heights, IL), according to the manufacturer's specifications. The probe was purified using a NUCTRAP push column (Stratagene, La Jolla, CA). ExpressHyb™ (Clontech) solution was used for prehybridization and as a hybridizing solution for the Northern blots. Hybridization took place overnight at 55oC using 2 x 106 cpm/ml of labeled probe. The blots were then extensively washed at room temperature in 2X SSC, 0.1% SDS, followed a wash at 65°C in 0.1X SSC, 0.1% SDS. One 2 kb transcript corresponding to zsig25 was detected in $CD8^+$, a weaker transcript was detected in $CD4^+$ cells.

Additional analysis was carried out on Northern blots made with poly(A) RNA from the from K-562 cells (erythroid, ATCC CCL 243), HUT78 cells (T cell, ATCC TIB-161), Jurkat cells (T cell), DAUDI (Burkitt's human lymphoma, Clontech, Palo Alto, CA), RAJI (Burkitt's human lymphoma, Clontech) and HL-60 (Promyelocytic human

leukemia, Clontech). Total RNA was prepared using a guanidine isothiocynate, phenol, chloroform mixture according to Cheomczynski and Sacchi (Anal. Biochem. 162:156-9, 1987). Poly(A)+ RNA was isolated using oligo d(T) cellulose chromatography (Aviv and Leder, Proc. Natl. Acad. Sci. U.S.A. 69: 1408-1412, 1972). Total RNA from $CD4^+$, $CD8^+$, $CD19^+$ and mixed lymphocyte reaction cells (CellPro, Bothell, WA) was prepared using guanidine isothiocyanate (Chirgwin et al., Biochemistry 18: 52-94, 1979) followed by a CsCl centrifugation step. Poly(A)+ RNA was isolated as above. Poly A+ RNA from DAUDI (Burkitt's human lymphoma, ATCC CCL213), RAJI (Burkitt's human lymphoma, ATCC CCL86), HL-60 (Promyelocytic human leukemia, ATCC CCL240) and MOLT-4 (Lymphoblastic human leukemia, ATCC CCL240) was purchased from Clontech (Palo Alto, CA). Northern blot analysis was then performed as follows.

About 2 µg of each of the poly A+ RNAs was denatured in 2.2 M formalahyde/phosphate buffer (50 mM Na_2HPO_4, 50 mM NaH_2PO_4, 50 mM NaOAc, 1 mM EDTA and 2.2 M formaldehyde) and separated by 1.5% agarose mini gel (Stratagene Cloning Systems, La Jolla, CA) electrophoresis in formaldehyde/phosphate buffer. The RNA was blotted overnight onto a nytran filter (Schleicher & Schuell, Keene, NH) and the filter was UV crosslinked (1,200 lJoules) in a STRATALINKER® UV crosslinker (Stratagene Cloning Systems) and then baked at 80°C for 1 hour. Hybridization was as described above. No transcripts were detected.

Additional analysis was carried out on fetal tissue Northern blots, Human Fetal II (Clontech) and Human Fetal Tissue Blots I and II (OriGene Technologies, Rockville, MD). Hybridization was as described above. A 2.0 kb transcript corresponding to zsig25 was detected in lung, liver and kidney tissue on the Clontech fetal blot. Two transcripts, 2.2 kb and 6.0 kb, corresponding to

zsig25 were detected in liver, lung, muscle, heart, kidney, skin and small intestine from the OriGene Technologies blots.

Example 3
Chromosomal Mapping

The zsig25 gene was mapped to chromosome 4 using the commercially available GeneBridge 4 Radiation Hybrid Panel (Research Genetics, Inc., Huntsville, AL). The GeneBridge 4 Radiation Hybrid Panel contains PCRable DNAs from each of 93 radiation hybrid clones, plus two control DNAs (the HFL donor and the A23 recipient). A publicly available WWW server (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl) allows mapping relative to the Whitehead Institute/MIT Center for Genome Research's radiation hybrid map of the human genome (the "WICGR" radiation hybrid map) which was constructed with the GeneBridge 4 Radiation Hybrid Panel.

For the mapping of the zsig25 gene with the GeneBridge 4 RH Panel, 20 μl reactions were set up in a 96-well microtiter plate (Stratagene) and used in a RoboCycler Gradient 96 thermal cycler (Stratagene). Each of the 95 PCR reactions consisted of 2 μl 10X KlenTaq PCR reaction buffer (Clontech), 1.6 μl dNTPs mix (2.5 mM each, PERKIN-ELMER, Foster City, CA), 1 μl sense primer, ZC 13,699 (SEQ ID NO. 18), 1 μl antisense primer, ZC 13,700 (SEQ ID NO. 19), 2 μl *Redi*Load (Research Genetics, Inc.), 0.4 μl 50X Advantage KlenTaq Polymerase Mix (Clontech Laboratories, Inc.), 25 ng of DNA from an individual hybrid clone or control and ddH_2O for a total volume of 20 μl. The reactions were overlaid with an equal amount of mineral oil and sealed. The PCR cycler conditions were as follows: an initial 1 cycle 5 minute denaturation at 95°C, 35 cycles of a 1 minute denaturation at 95°C, 1 minute annealing at 64°C and 1.5 minute extension at 72°C,

followed by a final 1 cycle extension of 7 minutes at 72°C. The reactions were separated by electrophoresis on a 3% NuSieve GTG agarose gel (FMC Bioproducts, Rockland, ME).

The results showed that the zsig25 gene maps 4.92 cR_3000 from the framework marker WI-6657 on the human chromosome 4 WICGR radiation hybrid map. Proximal and distal framework markers were WI-6657 (D4S2799) and WI-5430 (D4S2663), respectively. This positions the zsig25 gene in the 4p16.3 region on the integrated LDB chromosome 4 map (The Genetic Location Database, University of Southhampton, WWW server: http://cedar.genetics.soton.ac.uk/public_html/).

Of particular interest to note is that the critical region for the Wolf-Hirschhorn syndrome has also been mapped to this region (Wright et al., Hum. Mol. Genet. 6: 317-24, 1997). The article describes a transcript map of the newly defined 165 kb Wolf-Hirschhorn syndrome critical region. A cDNA clone described in this paper (27812, SEQ ID NO:37) was mapped as described above using oligonucleotide primers ZC17143 (SEQ ID NO:25) and ZC17144 (SEQ ID NO:36). Zsig25 and the cDNA clone 27812 were found to map equidistant from the proximal framework marker WI-6657.

Example 4

Construction of zsig25 Mammalian Expression Vectors zSIG25CF/pZP9 and zSIG25NF/pZP9

Two expression vectors were prepared for the zsig25 polypeptide, zSIG25CF/pZP9 and zSIG25NF/pZP9, wherein the constructs are designed to express a zsig25 polypeptide with a C- or N-terminal FLAG tag (SEQ ID NO: 20).

zSIG25CF/pZP9

A 1032 bp PCR generated CF/ZSIG-25 DNA fragment was created using ZC13,660 (SEQ ID NO:21) and ZC13661 (SEQ ID NO:22) as PCR primers and the template described in

Example 1 above. The PCR reaction was incubated at 94°C for 5 minutes, and then run for 10 cycles of 30 seconds at 94°C and 2 minutes at 72°C, followed by 15 cycles at 94°C for 30 seconds and 65°C for 2 minutes. The resultant PCR product was then run on a 0.9% GTG/TBE agarose gel with 1x TBE buffer. A band of the predicted size, 1032 bp, was excised and the DNA was purified from the gel with a QUIAQUICK® column (Qiagen) according the manufacturer's instructions. The DNA was digested with the restriction enzymes BAM HI (Boehringer Mannheim) and Eco RI (Gibco BRL), followed by phenol/chloroform/isoamyl alcohol extraction and precipitated.

The excised DNA was subcloned into plasmid CF/pZP9 which had been cut with Eco RI and Bam HI. The zSIG25/CFpZP9 expression vector uses the native zSIG25 signal peptide, and the FLAG epitope (SEQ ID NO:20) is attached at the C-terminus as a purification aid. Plasmid CF/pZP9 (deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD) is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 promoter, multiple restriction sites for insertion of coding sequences, a sequence encoding the flag peptide (SEQ ID NO:20), a stop codon and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

zSIG25NF/pZP9

A 954 bp PCR generated zSIG25/NF DNA fragment was created in accordance with the procedure set forth above using Z13658 (SEQ ID NO:23) and ZC13659 (SEQ ID NO:24) as PCR primers. The purified PCR fragment was digested with the restriction enzymes BAM HI (Boehringer

Mannheim) and Xho I (Gibco BRL), followed by DCI extraction and ETOH/glycogen precipitated.

The excised zSIG25/NF DNA was subcloned into plasmid NF/pZP9 which had been cut with Bam HI and Xba I. The zSIG25/NFpZP9 expression vector incorporates the TPA leader and attaches the FLAG epitope (SEQ ID NO:20) to the N-terminal of the zsig25 polypeptide-encoding polynucleotide sequence. Plasmid NF/pZP9 (deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD) is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 promoter, a TPA leader peptide followed by the sequence encoding the FLAG peptide (SEQ ID NO:20), multiple restriction sites for insertion of coding sequences, and a human growth hormone terminator. The plasmid also contains an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator.

Ten nanograms of the restriction digested C- and N-terminal FLAG/zsig25 inserts and 20 ng of the corresponding vectors were ligated at room temperature for 4 hours. One microliter of each ligation reaction was independently electroporated into DH10B competent cells (GIBCO BRL, Gaithersburg, MD) according to manufacturer's direction and plated onto LB plates containing 50 mg/ml ampicillin, and incubated overnight. Colonies were screened by PCR as described above. For zSIG25CF/pZP9 screens the primers were, ZC13660 (SEQ ID NO:21) and ZC13661 (SEQ ID NO:22) and for zSIG25NF/pZP9 screens the primers were ZC13658 (SEQ ID NO:23) and ZC13659 (SEQ ID NO:24). The insert sequence of positive clones, 954 bp fragment for zSIG25NF and a 1032 bp fragment for zSIG25/CF were verified by sequence analysis. A large scale plasmid preparation was done using a QIAGEN® Maxi prep kit (Qiagen) according to manufacturer's instructions.

Example 5

Expression of zsig25

Mammalian

BHK 570 cells (ATCC No. CRL-10314) were plated in 10 cm tissue culture dishes and allowed to grow to approximately 50 to 70% confluency overnight at 37°C, 5% CO_2, in DMEM/FBS media (DMEM, Gibco/BRL High Glucose, (Gibco BRL, Gaithersburg, MD), 5% fetal bovine serum (Hyclone, Logan, UT), I μM L-glutamine (JRH Biosciences, Lenexa, KS), 1 μM sodium pyruvate (Gibco BRL)). The cells were then transfected with the plasmid zSIG25NF/pZP9 (N-terminal FLAG tag) or zSIG25CF/pZP9 (C-terminal FLAG tag), using Lipofectamine™ (Gibco BRL), in serum free (SF) media formulation (DMEM, 10 mg/ml transferrin, 5 mg/ml insulin, 2 mg/ml fetuin, 1% L-glutamine and 1% sodium pyruvate). Sixteen micrograms of zSIG25NF/pZP9 and 16 μg of zSIG25CF/pZP9 were separately diluted into 15 ml tubes to a total final volume of 640 μl SF media. In separate tubes, 35 μl of Lipofectamine™ (Gibco BRL) was mixed with 605 μl of SF medium. The Lipofectamine™ mix was added to the DNA mix and allowed to incubate approximately 30 minutes at room temperature. Five milliliters of SF media was added to the DNA:Lipofectamine™ mixture. The cells were rinsed once with 5 ml of SF media, aspirated, and the DNA:Lipofectamine™ mixture was added. The cells were incubated at 37°C for five hours, then 6.4 ml of DMEM/10% FBS, 1% PSN media was added to the plate. The plate was incubated at 37°C overnight and the DNA:Lipofectamine™ mixture was replaced with fresh FBS/DMEM media the next day. On day 2 post-transfection, the cells were split into the selection media (DMEM/FBS media from above with the addition of 1 μM methotrexate (Sigma Chemical Co., St. Louis, Mo.)) in 150 mm plates at 1:10, 1:20 and 1:50. The

plates were refed at day 5 post-transfection with fresh selection media.

Screening colonies

zSIG25NF/pZP9

Approximately 10 days post-transfection, two 150 mm culture dishes of methotrexate resistant colonies were trypsinized and the cells were pooled. Cells were plated on to five 96 well plates each, at concentrations of 0.5 cells/well and 1 cell/well. The cells were grown to confluence and a set of five plates at the optimum density of 30 colonies/plate was selected for ELISA analysis.

The media was aspirated from these plates and replaced with 140 µl/well serum free ESTEP 2 media (668 g/l DMEM, 5.5 g/l pyruvic acid, sodium salt, 185 g/l $NaHCO_3$, 25 ml/l insulin, 25 ml/l transferrin, pH 7.05). The conditioned media was harvested at 72 hours and transferred to a fresh set of 96 well plates, referred to as sample plates. The sample plates were covered with Plate Sealers (Costar, Pleasanton, CA) and placed at -80 ^oC until time of assay. The cells in the culture plates were fed DMEM/5% FBS media containing 1 µM MTX and kept in the incubator until the analysis was complete.

A competition ELISA was set up as follows. Using the TomTek Quadra 96 robot to prepare plates for assay, 100 µl/well of 250 ng/ml IL-4sFLAG (FLAG tagged soluble portion of human IL-4) in 0.1 M NaH_2CO_4 was pipetted into twelve 96 well plates (Maxisorb, Nunc AS, Roskilde, Denmark). These are referred to as assay plates. The assay plates were incubated overnight at 4^oC. The excess IL-4sRFLAG solution was removed and the plates were blocked in 10 mg/ml BSA, 0.05% Tween 20 in PBS for one hour at room temperature.

A standard plate was prepared by setting up a two fold serial dilution in each plate in duplicate, across eleven columns, from 1000 ng/ml to 0.97 ng/ml in 140 µl of media, and a media blank in the twelfth column.

The five sample plates were thawed and 72 µl/well of 667 µg/ml anti-FLAG M2 monoclonal antibody (Kodak) was added to the sample and standard plates. These were incubated at room temperature for 30 minutes.

The application of the sample and standards to assay plates was performed with the computer-programmed SCITECH ORCA Robot are system (manufacturer). The assay plates were washed for times with 0.05% Tween 20 in PBS. The standards and samples were each split between two assay plates, 75 µl/well, for duplicate assays. The assay plates were incubated 30 minutes at room temperature.

The ORCA robot was used for development and analysis of the assays. OPD reagent was made immediately prior to use by dissolving 1 OPD tablet (o-phenylenediamine dihydrochloride, Sigma Chemical Co., St. Louis, Mo.) into 12 ml of NOVO D reagent (313 ml 0.1 M sodium citrate added to 187 ml 0.1 M citric acid). The assay plates were washed four times with 0.5% Tween 20 in PBS and 100 µl/well of OPD reagent was then added. The plates were allowed to incubate approximately 5 minutes at room temperature and then stopped with 100 µl of 1N N_2SO_4. The 490 nm absorbance was read in an ELISA plate reader (SLT.Spectra 96pw).

The five highest yielding clones were picked from the culture dishes and transferred to 12 well plates and raised to confluence. The media was replaced with serum free ESTEP2 and the conditioned media was harvested for Western blot analysis. The cells were transferred to T-75 flasks in DMEM/5% FBS, 1 µM MTX media. One flask from each clone is grown in serum-free ESTEP 2 and the media harvested for Western Blot analysis. The three best clones of zSig25NF, based on Western blot analysis were selected, pooled together and transferred to large scale culture.

ZSIG25CF/pZP9

Approximately 10-12 days post-transfection, two 150 mm culture dishes of methotrexate resistant colonies were chosen, the media aspirated, the plates washed with 10 ml serum-free ESTEP 2 media. The wash media was aspirated and replaced with 5 ml serum-free ESTEP 2. Sterile Teflon mesh (Spectrum Medical Industries, Los Angeles, CA) pre-soaked in serum-free ESTEP 2 was then placed over the cells. A sterile nitrocellulose filter pre-soaked in serum-free ESTEP 2 was then placed over the mesh. Orientation marks on the nitrocellulose were transferred to the culture dish. The plates were then incubated for 5-6 hours in a 37°C, 5% CO_2 incubator. Following incubation, the filter was removed, and the media aspirated and replaced with DMEM/5% FBS, 1X PSN (Gibco BRL) media. The filters were blocked in 2.5% nonfat dry milk/Western A buffer (Western A: 50mM Tris pH 7.4, 5 mM EDTA, 0.05% NP-40, 150 mM NaCl and 0.25% gelatin) overnight at 4°C on a rotating shaker. The filter was then incubated with a goat anti-human FLAG-HRP conjugate at a 1:4000 dilution (5 µl antibody in 20 ml buffer) in 2.5% nonfat dry milk/Western A buffer (Western A: 50mM Tris pH 7.4, 5 mM EDTA, 0.05% NP-40, 150 mM NaCl and 0.25% gelatin) at room temperature for 1 hour on a rotating shaker. The filter was then washed three times at room temperature in PBS plus 0.1% Tween 20, 15 minutes per wash. The filter was developed with ECL reagent (Amersham Corp., Arlington Heights, IL) according the manufacturer's directions and exposed to film (Hyperfilm ECL, Amersham) for approximately 5 minutes.

The film was aligned with the plate containing the colonies. Using the film as a guide, suitable colonies were selected. Sterile, 3 mm coloning discs (PGC Scientific Corp., Frederick, MD) were soaked in trypsin, and placed on the colonies. The colonies were transferred into 200 µl of selection medium in a 96 well plate. A series of seven, two-fold dilutions were carried out for

each colony. The 150 mm culture dish was then trypsinized and the remainder of the cells are pooled and split into two T162 flasks containing DMEM/5% FBS and 1 μM MTX media. The cells were grown for one week at 37°C at which time the wells which received the lowest dilution of cells which are now at the optimum density were selected, trypsinized and transferred to a 12 well plate containing selection media.

The clones were expanded directly from the 12 well plate to 2 T-75 flasks. One flask from each clone is grown in serum-free ESTEP 2 and the media harvested for Western Blot analysis. The three best clones of zSig25CF, based on Western blot analysis were selected, pooled together and transferred to large scale culture.

Example 6

Large Scale Culture of zsig25 FLAG tagged polypeptides

One T-162 flask, containing confluent cells expressing zsig25/CF and zsig25/NF obtained from the expression procedure described above, were expanded into six T-162 flasks. One of the six resulting flasks was used to freeze down four cryovials, and the other five flasks were used to generate a Nunc cell factory.

The cells from the five T-165 flasks were used to seed a Nunc cell factory (10 layers, commercially available from VWR). Briefly, the cells from the T-162 flasks described above were detached using trypsin, pooled and added to 1.5 liters ESTEP 1 media (668.7g/50L DMEM (Gibco), 5.5 g/50L pyruvic acid, sodium salt 96% (Mallinckrodt), 185.0 g/50 L $NaHCO_3$ (Mallinkrodt), 5.0 mg/ml and 25 ml/50 L insulin (JRH Biosciences), 10.0 mg/ml and 25 ml/50 L transferrin (JRH Biosciences), 2.5L/50L fetal bovine serum (characterized) (Hyclone), 1 μM MTX, pH 7.05) prewarmed to 37°C. The media containing cells was

poured into a Nunc cell factory via a funnel. The cell factory was placed in a 37°C, 5.0% CO_2 incubator.

At 80-100% confluence, a visual contamination test (phenol red color change) was performed on the Nunc cell factory. Since no contamination was observed, supernatant from the confluent factory was poured into a small harvest container, sampled and discarded. The adherent cells were then washed once with 400 ml PBS. To detach the cells from the factory, 100 mls of trypsin was added and removed and the cells were then incubated for 5 to 10 minutes in the residual trypsin. The cells were collected following two, 200 ml washes of ESTEP1 media. Forty milliliters of collected cells were then used to seed each of ten Nunc cell factories. To ten ESTEP1 media-containing bottles (1.5 liters each, at 37°C) was added 40 mls of collected cells. One 1.5 liter bottle was then used to fill one Nunc factory. Each cell factory was placed in a 37°C/5.0% CO_2 incubator.

At 80-90% confluence, a visual contamination test (phenol red color change) was performed on the Nunc cell factory. Since no contamination was observed, supernatant from the confluent factory was poured into a small harvest container, sampled and discarded. Cells were then washed once with 400 ml PBS. ESTEP2 media (1.5 liters, 668.7g/50L DMEM (Gibco), 5.5 g/50L pyruvic acid, sodium salt 96% (Mallinckrodt), 185.0 g/50L $NaHCO_3$ (Mallinkrodt), 5.0 mg/ml, 25 ml/50L insulin, 10.0 mg/ml and 25 ml/50 L transferrin) was added to each Nunc cell factory. The cell factory were incubated at 37°C/5.0% CO_2.

At approximately 48 hours (zsig25/NF, 15 L was obtained) and 72 hours (zsig25/CF, 15 L was obtained), a visual contamination test (phenol red color change) was performed on the Nunc cell factories. Supernatant from each factory was poured into small harvest containers. Fresh serum-free ESTEP 2 media (500 ml) was poured into each Nunc cell factory, and the factories were incubated

at37°C/5.0% CO_2. One ml of supernatant harvest was transferred to a microscope slide, and subjected to microscopic analysis for contamination. The contents of the small harvest containers for each factory were pooled and immediately filtered. A second harvest was then performed, substantially as described above at 46 hours (zsig25/NF, 13.5 L were obtained) and 72 hours (zsig25/CF, 13.5 L were obtained) and the cell factories were discarded thereafter. An aseptically assembled filter train apparatus was used for aseptic filtration of the harvest supernatant (conditioned media). Assembly was a follows: tubing was wire-tied to an Opti-Cap filter (Millipore Corp., Bedford, MA) and a Gelman Supercap 50 filter (Gelman Sciences, Ann Arbor, MI). The Supercap 50 filter was also attached to a sterile capped container located in a hood; tubing located upstream of the Millipore Opti-cap filter was inserted into a peristaltic pump; and the free end of the tubing was placed in the large harvest container. The peristaltic pump was run between 200 and 300 rpm, until all of the conditioned media passed through the 0.22 μm final filter into a sterile collection container. The filtrate was placed in a 4 °C cold room pending purification.

Concentration and Western Blot

Conditioned media containing zsig25/CF and zsig25/NF was collected for concentration at various time points (at the 5 T-162 flask stage; 1 factory, fetal bovine serum media; 10 factories, fetal bovine serum media; 10 factories, serum free media and a second 10 factory, serum free media time point). Since the expected mass of the protein was in excess of 8 kDA, Millipore 5 kDa cut off concentrators were used. The starting volume for each sample was 15 ml, which was concentrated to a final volume of 1.5 ml. The concentrators were spun at 4°C

in Beckman tabletop centrifuge at 2000 x g (3000 rpm) for 40 minutes. The concentrate was transferred to a 1.5 ml non-stick microfuge tube, and the volume was adjusted to 1 ml using flow through media to achieve a 10x concentration. To sterilize the media, the 10x concentrate was split into two Costar Spin-X tubes, and the tubes were spun at 8000 x g for two minutes in a Eppendorf 5415 microfuge (VWR, Seattle, WA).

Western blot analysis was also conducted for the zsig25/CF and zsig25/NF samples described above. NuPage gel electrophoresis is conducted using 25 µl of conditioned media and 25 µl 2X reducing sample buffer, according to manufacturer's instructions, running the get at 150 volts for approximately one hour. The conditioned media sample lanes were loaded with 32.5 µl of sample. Following electrophoresis, the gels were transferred to 2 µm supported nitrocellulose (BioRad) at room temperature for 1 hour (500 mA) using a Hoeffer transfer tank unit (Hoeffer Scientific Instruments, San Francisco, CA) with stirring in accordance with the manufacturer's instructions. The transfer buffer contained 25 mM Tris-Base, 200 mM glycine, and 20% MeOH. Next, the nitrocellulose filters were blocked for 10 minutes at room temperature with 10% non-fat dry milk (NFDM) in Western A buffer (50 mM Tris, pH 7.4; 5 mM EDTA solution, pH 8.0; 0.05% Igepal (Sigma); 150 mM NaCl and 0.25% gelatin). The membrane was then rinsed with Western A buffer. The primary antibody, α-FLAG M2 (Kodak) was added at 0.5 ug/ml in Western A buffer containing 2.5% NFDM with shaking or rocking overnight at 4°C. The membrane was then washed three times for 5 minutes in Western A buffer. A secondary antibody, goat α-mouse IgG-HRP (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was added in Western A buffer containing 2.5% NFDM (10 µl of 400 µg/ml antibody solution in 20 mls Western A for a 1:2000 dilution) with shaking or rocking for one hour at room temperature. The

membrane was then washed three times for 5 minutes in Western A buffer, and then rinsed in Milli-Q water. The membrane was then placed into a sheet protector (Avery Office Products, Gold Bar, CA). A 1:1 solution of ECL Western Blotting Detection Reagents (Amersham Life Science., Buckinghamshire, England) was mixed and 500 µl of solution was added to the left edge of the membrane. The solution was then slowly spread over the blot and excess reagent was removed after one minute. The blot was then exposed to ECL Hyperfilm 8x10 (Amersham Corp., Arlington Heights, IL) for 10 seconds and 30 seconds.

The results of concentration and Western Blot analysis provided the following concentration and molecular weight estimates:

zSIG25/CF:
5 T-162 Flasks = 0.27 mg/L, 36 kDa;
1 Factory, FBS = 0.27 mg/L, 36 kDa;
10 Factories, FBS = 0.27 mg/L, 36 kDa;
10 Factories (#1), SF = 0.95 mg/L, 38 kDa; and
10 Factories (#2), SF = 0.95 mg/L, 38 kDa.

zSIG25/NF:
5 T-162 Flasks = no protein detected;
1 Factory, FBS = 0.49 mg/L, 38 kDa;
10 Factories, FBS = 0.49 mg/L, 38 kDa;
10 Factories (#1), SF = 1.46 mg/L, 38 kDa; and
10 Factories (#2), SF = 1.46 mg/ml, 38 kDa, second band at 28 kDa.

Example 7
Purification and Analysis of zsig25/NF and zsig25/CF

All of the procedures are carried out at 4°C, unless otherwise noted. A total of 25 liters of conditioned zSIG25CF and zSIG25NF media from above was sequentially sterile filtered through a 4 inch, 0.2 µM Opti-cap filter (Millipore Corp., Bedford, MA) and a 0.2

μM Supercap 50 filter (Gelman Sciences, Ann Arbor, MI). The material was then concentrated to about 1.3 liters using an Amicon DC 10L concentrator (Amicon, Beverly, MA) fitted with an A/G Tech hollow fiber cartridge (A/G Tech, Needham, MA) with a 15 sq. ft. 3000 kDa cutoff membrane. The concentrated material was again sterile filtered with a Gelman filter as described above. A 25 ml aliquot of anti-FLAG Sepharose (Kodak) was added to the concentrated material for batch absorption and the mixture was gently agitated on a Wheaton roller culture apparatus (Wheaton, Millville, NJ) for 18 hours at 4°C.

The Sepharose mixture was then poured into a 5.0 x 20.0 cm Econo-Column (Bio-Rad Laboratories, Hercules, CA) and the gel was washed with 30 column volumes of phosphate buffered saline (PBS). The unretained flow-through fraction was discarded. Once the absorbance of the effluent at 280 nM was less than 0.05, flow through the column was reduced to zero and the anti-FLAG Sepharose gel was washed with 2.0 column volumes of PBS containing 0.2 mg/ml of FLAG peptide (SEQ ID NO:20). After 1.0 hour at 4°C, flow was resumed and the eluted protein was collected. This fraction is referred to as the peptide elution. The anti-FLAG Sepharose gel was washed with 2.0 column volumes of 0.1 M glycine, pH 2.5, and the glycine wash was collected separately. The pH of the glycine-eluted fraction was adjusted to 7.0 by the addition of a small volume of 10X PBS and stored at 4°C.

The peptide elution was concentrated at 5.0 ml using a 5,000 molecular weight cutoff membrane concentrator (Millipore) according the manufacturer's instructions. The concentrated peptide elution was then separated from free peptide by chromatography on a 1.5 x 50 cm Sephadex G-50 (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) column equilibrated in PBS at a flow rate of 1.0 ml/minute using a BioCad Sprint HPLC system (PreSeptive BioSystems, Farmington, MA). Two milliliter

fractions were collected and the absorbance at 280 nM was monitored. The first peak of material absorbing at 280 nM and eluting near the void volume of the column was collected.

SDS-PAGE and Western Blot analysis using anti-FLAG M2 antibodies (Kodak) was done on the purified material. Purified zSIG25NF was composed of approximately equimolar amounts of two Coomassie Blue-stained bands of apparent molecular weights 30,000 and 41,000, that also showed cross-reactivity with the anti-FLAG M2 monoclonal antibody. Each band exhibited slightly greater mobility on the SDS-PAGE gels in the absence of reducing agents. Purified zSIG25CF was composed of a single Commassie Blue-stained band of apparent molecular weight 41,000 that also cross-reacted with the anti-FLAG antibodies. This material also showed slightly greater mobility under non-reducing conditions on SDS-PAGE gels.

The protein concentration of the purified proteins was determined by BCA analysis (Pierce, Rockford, IL) according to the manufacturer's instructions. The concentration of zSIG25CF was 1.05 mg/ml and zSIG25NF was 1.08 mg/ml. N-terminal sequence analysis and amino acid analysis confirm the identity of the purified material.

Example 8
Proliferation Assays

The ability of zsig-25 to stimulate proliferation of factor dependent hematopoietic cell lines was assayed as follows. A series of dilutions were prepared from both N- and C-terminal FLAG-tagged (NF or CF) zsig25, from 10 to 0.078 µg/ml. The dilutions were prepared at 2 times the final concentration in base medium (RPMI 1640, 2 mM L-glutamine, 110 µg/ml sodium pyruvate, PSN and 10% heat inactivated fetal bovine serum) and plated into a 96 well plate (Costar, Pleasanton, CA) at a

final volume of 100 µl/well. Hematopoietic factor dependent cell lines were washed with base medium. The cells were resuspended in testing media (base medium supplemented with sub-optimal concentration of the appropriate cytokine (1 pg/ml murine IL-3, 50 pg/ml murine IL-2, 10 pg/ml murine IL-7 or 1-5 ng/ml murine stem cell factor (SCF)) at two times the final assay concentration). The cells were plated into the above 96 well plates at the densities of between 5,550 to 10,000 cells/well in 100 µl test media. A duplicate plate was prepared without the cytokine supplements. Also included were control wells where the cells received neither cytokine nor zsig25. The cells were incubated at 37°C under 5% CO_2 for 3 to 6 days. The cells were then visually inspected and scored by eye for stimulation or inhibition of cell proliferation.

A dye incorporation assay was also used to quantitatively measure the proliferation of cells based on a colorimetric change and an increase in fluorescent signal. Twenty µl/well of alamar Blue™ (AccuMed, Chicago, IL) was added to the 96 well plates and the cells were incubated at 37°C under 5% CO_2 for 15-24 hours. The plates were then read using a fluorometer with excitation wavelength of 544 nm and emission wavelength of 590 nm.

To further measure the effect of NF- and CF-zsig25 on cell proliferation a 1° Marrow assay was also done. Bone marrow was harvested, by aspiration, from the femurs of 6-8 week old, euthanized, male BALB/C mice. Low density, mononuclear cells were selected for by centrifugation on a density gradient. Whole bone marrow was suspended in 5-7 ml of base medium which was added to the top of 6 ml Nycoprep (1.077, Animal, GIBCO BRL, Gaithersburg, MD) and centrifuged at 1,500 RPM (~600 x g) in a Beckman table-top centrifuge for 20 minutes. The mononuclear cells, a cloudy interface between the media and the Nycoprep layer, was removed and washed in 20-40 ml base media. The mononuclear cells were then pelleted by

centrifugation (5 minutes, 1400 rpm) and resuspended at a concentration of 1 x 10^6 cells/ml in base medium. The cells were then plated into a T 75 flask and incubated for 2 hours at 37°C, 5% CO_2. Non-adherent marrow cells were harvested and plated in a 96 well plate at a density of 40,000 cells/well. A series of 2X dilutions, from 10 to 0.078 µg/ml, were prepared from both NF- and CF-zsig25 in base medium alone, or media supplemented with 25 ng/ml mSCF or 5 ng/ml mIL-7 and added to the cells. The cells were incubated at 37°C under 5% CO_2 for 2 to 6 days. The cells were scored for stimulation or inhibition of proliferation, both visually and by change in fluorescence using alamar Blue as described above.

A bone marrow clonogenic assay was also performed. Bone marrow cells were prepared as described above. For each sample, the bone marrow cells were resuspended at 10x of the final desired concentration, 50,000 cells/ml, in RPMI media. The cells were then added to methyl cellulose growth media containing 10 ng/ml IL-7 (cat#HCC-3630, Stem Cell Technologies, Vancouver, British Columbia, Canada) in a 1:9 vol:vol ratio. Zsig25 was added to the cells at a final concentration of 200, 600 or 1800 ng/ml. IL-1 was included as a control at 1800 ng/ml. A zsig25/IL-1 negative sample was also tested. The sample proteins were added to the cell solutions and gently mixed for about 2 minutes. To duplicate wells of a 6 well plate was added 1 ml of the sample/cell solution. The cells were incubated at 37°C, 5% CO_2 until colonies appeared. Colonies were scored between day 7 and day 11.

NF-zsig25 had a stimulatory effect whereas no response was seen with CF-zig25. NF-zsig25 stimulated proliferation of BaF3, BaF3 pZR103 #2 and DA-1 cell lines. BaF3 is an interleukin-3 dependent pre-lymphoid cell line derived from murine bone marrow (Palacios and Steinmetz, Cell 41: 727-34, 1985; Mathey-Prevot et al., Mol. Cell. Biol. 6: 4133-5, 1986). BaF3 pZR103 #2, (described

below), is a BaF3 cell line expressing a zcytor-1/mpl hybrid receptor which exhibits a greater sensitivity to IL-4 and IGF-I than does the parent BaF3 line (Figure 3). DA-1, an IL-3 dependent cell line derived from the lymph node of a mouse with a B-cell lymphoma by outgrowth in IL-3 containing media (provided by Kenneth Kaushansky, University of Washington, Seattle, WA)(Figure 4). The stimulatory effect of NF-zsig25 on these cell lines was visible by eye and by a increase in the amount of fluorescence, down to the 250 ng/ml range, when compared to controls which did not receive NF-zsig25.

Stimulation in the 1° Marrow assay was seen after 2-6 days when co-stimulated with mIL-7 at 5-10 ng/ml (Figure 6). Figure 5 shows stimulation of 1° Marrow with murine IL-3.

Colony formation was stimulated by zsig25. The number of colonies nearly doubled for the cells treated with 1800 ng/ml zsig25 compared to the negative control. No growth stimulation was seen at the 200 ng/ml dose and a slight increase over background was seen at the 600 ng/ml dose.

BaF3 pZR103 #2 (pZR103-gp130 BaF3 #2) contains a chimeric cytokine receptor having the extracellular domain of mouse Zcytor1 (WIPO Publication No. 97/44455, SEQ ID NO:26) and the transmembrane and cytoplasmic domains of the mouse MPL receptor (Skoda et al., EMBO J. 12: 2645-2653, 1993). This construct was introduced into BaF3 cells expressing gp130.

An expression vector encoding a mouse Zcytor1-MPL chimeric receptor was constructed. The chimera comprised the extracellular domain of Zcytor1 fused at its C-terminus (residue 1-508 of SEQ ID NO:27) to the transmembrane domain of the mouse MPL receptor (residue 490-633 of SEQ ID NO:28).

An extracellular mouse Zcytor1 DNA fragment was prepared by PCR using 40 pmol of oligonucleotide primers

ZG10301 (SEQ ID NO:28) and ZG10302 (SEQ ID NO:29). The primers were combined with 100 ng of template DNA (mouse Zcytor1 expression vector T1323D, WIPO Publication No. 97/44455), 5 µl of 2.5 mM dNTPs (Perkin-Elmer Corp.), 5 µl of 10X buffer (KlenTaq PCR buffer, Clontech), 1 µl of KlenTaq DNA polymerase (Clontech), and 34 µl H_2O. The reaction was run for 20 cycles of 94°C, 1 minute; 45°C, 1 minute; and 72°C, 1 minute; followed by a 7 minute extension at 72°C. The resulting fragment continues a 3' sequence encoding amino acid residues WITLVTA (SEQ ID NO:30) which overlap with the mouse MPL sequence

A transmembrane and cytoplasmic domain containing mouse MPL receptor fragment was prepared by PCR as described above, using 40 pmol of oligonucleotides ZC10297 (SEQ ID NO:31) and ZC10298 (SEQ ID NO:32) and 100 ng template DNA (mouse MPL receptor expression vector, WIPO Publication No. 95/21920). The resulting fragment contains a 5' sequence encoding amino acid residues PDNRIR (SEQ ID NO:33) which overlap with the mouse Zcytor1 sequence.

The reaction products were visualized by agarose gel electrophoresis and the sizes confirmed, 430 bp mouse Zcytor1 fragment and 460 bp mouse MPL fragment.

A second PCR was performed to join the Zcytor1 and MPL fragments at the overlap encoding the amino acid sequence PDNRIRWITLVTA (SEQ ID NO: 34) using 40 pmol oligonucleotide primers ZC10302 (SEQ ID NO:29) and ZC10297 (SEQ ID NO:31). The primers were added to 1 µl template (a mixture of 3 µl of each of the above PCR reaction products), 5 µl of 2.5 mM dNTPs (Perkin-Elmer Corp.), 5 µl of 10X buffer (KlenTaq PCR buffer, Clontech), 1 µl of KlenTaq DNA polymerase (Clontech), and 31 µl H_2O. The reaction was run for 20 cycles of 94°C, 1 minute; 45°C, 1 minute; and 72°C, 1 minute; followed by a 7 minute extension at 72°C. A band of the expected size, ~890 bp, was visualized by gel electrophoresis and the remaining

PCR fragment was purified using a Wizard® DNA Clean UP kit (Promega Corp., Madison, Wi.) according to manufacturer's instruction. The PCR fragment was then and cut with restriction enzymes Apa I and Xho I. A band of the expected size, ~520 bp, was visualized by 1% agarose gel electrophoresis, excised and purified.

To construct an expression vector for the mouse Zcytor1-MPL fusion, pHZ-1 was digested with Eco RI and Xho I. Plasmid pHZ-1 is an expression vector that may be used to express protein in mammalian cells or in a frog oocyte translation system from mRNAs that have been transcribed *in vitro*. The pHZ-1 expression unit comprises the mouse metallothionein-1 promoter, the bacteriophage T7 promoter flanked by multiple cloning banks containing unique restriction sites for insertion of coding sequences, the human growth hormone terminator and the bacteriophage T7 terminator. In addition, pHZ-1 contains an *E. coli* origin of replication; a bacterial beta lactamase gene; a mammalian selectable marker expression unit comprising the SV40 promoter and origin, a neomycin resistance gene and the SV40 transcription terminator.

The linearized vector was ligated to the mouse Zcytor1/MLP PCR Apa I/Xho I fragment from above and an Eco RI/Apa I mouse Zcytor1 fragment to complete the 5' end of the chimera. To the ligation mix was added 3 µl of the vector, 5 µl of each fragments, 4 µl 5X ligation buffer and 1 µ ligase. The ligation proceeded for 6 hours at room temperature followed by electroporation of 1 µl of the ligation mix into Ca^{++} competent E. coli DH10B-cells. Colonies were screened by PCR using oligonucleotide primers ZC10302 (SEQ ID NO:29) and ZC10297 (SEQ ID NO:31) as described above, with 30 cycles of 94°C, 1 minute; 55°C, 1 minute; and 72°C, 1 minute; followed by a 7 minute extension at 72°C. The sequence of positive clones was verified by sequence analysis.

The mouse Zcytor1/MPL fusion construct was transfected into a BaF_3-gp130 cell line by electroporation. Transfectants were cultured in RPMI media supplemented with 10% fetal bovine serum, IL-3, Zeosin and G418.

The BaF_3-gp130 cell expressed the mouse b-subunit gp130 (Hibi et al., Cell 63:1149-57, 1990) which associates with receptor subunits specific for IL-6, IL-11, and LIF (Gearing et al., EMBO J. 10:2839-48, 1991; Gearing et al., U.S. Patent No. 5,284,755), under selection by zeosin. The cell line was generated by electroporating an expression vector containing gp130 into wild type BaF3 cells, under selection of zeosin and the IL-6 ligand and soluble IL-6 receptor.

Example 9

Adenovirus production of zsig25 NF and CF

Production of adenovirus containing zsig25NF and CF was done according to the procedure of Becker et al., Meth. Cell Biol. 43:161-89, 1994. Briefly, the cDNA encoding zsig25NF or zsig25CF was excised by Eco RI/Xba I restriction digest from zSIG25NF/pZP9 and zSIG25CF/pZP9. Restriction fragments were visualized by gel electrophoresis, excised and purified. The zsig25NF and CF restriction fragments were independently ligated into an Eco RI/Xba I digested shuttle vector, pAC-CMV (Microbix Biosystems, Inc., Ontario, Canada). One microliter of each ligation reaction was independently electroporated into DH10B competent cells (GIBCO BRL) according to manufacturer's direction and plated onto LB plates containing 50 mg/ml ampicillin, and incubated overnight. Colonies were screened by restriction digest and large scale plasmid DNA was prepared for positive clones.

The zsig25 containing shuttle vectors, zsig25NF/pAC-CMV and zsig25CF/pAC-CMV, were co-transfected with E1-deleted, adenovirus vector pJM17 (Microbix Biosystems, Inc.) into 293 cells (ATCC CRL-1573) which

express the adenovirus E1 gene. The co-transfection was done using a Transfection MBS Mammalian Transfection Kit (Stratagene Cloning Systems, La Jolla, CA), according to the manufacturer's instructions. Virus propagation is conditional and is achieved only by growing the E1-deleted virus in a cell line expressing the E1 gene. Recombinant virus is generated by homologous recombination of overlapping fragments of the viral genome in the pJM17 vector and the shuttle vector.

Cells were maintained for 2-4 weeks until the recombination event occurred. At that time, the host 293 cells were lysed by the virus, forming plaques of dead cells. Within 3-5 days the entire monolayer was completely lysed. The medium containing the viral lysate was collected and any remaining intact cells were lysed by repeated freeze/thaw cycles and the cell debris pelleted by centrifugation.

The viral lysate was then plaque purified according to the method of Becker et al., ibid. Briefly, serial dilutions were prepared in DMEM containing 10% fetal bovine serum and 100 U/ml penicillin/streptomycin, plated on to monolayers of 293 cells and incubated at 37°C for one hour. A melted 1.3% agarose/water solution was mixed with 2X DMEM (containing 4% FBS, 200 U/ml penicillin/streptomycin, 0.5 µg/ml fungizone and 30 mg/ml phenol red) and 6 ml was added to the virus infected 293 cells followed by incubation at 37 °C until plaques formed, 7-10 days. Single plaques were isolated and the presence of the zsig25 insert was verified by PCR. For zsig25NF/pAC-CMV and zsig25CF/pAC-CMV the primers were ZC12700 (SEQ ID NO:55) and ZC12742 (SEQ ID NO:57); and ZC12700 (SEQ ID NO:55) and ZC8947 (SEQ ID NO:56). Amplification was carried out over 30 cycles of 94°C, 1 minute; 55°C, 1 minute 30 seconds; and 72°C, 2 minutes; followed by a 10 minute extension at 72°C. The expected size of the PCR generated fragments were 1324 and 1434 bp

for zsig25NF and 1384 and 1494 bp for zsig25CF. The identity of the inserts was verified by sequence analysis.

One plaque from each construct was used to do a primary amplification according to the methods of Becker et al., ibid. Briefly, 30 dishes (150 x 25 mm) containing 293 cells at 80% confluence were infected at a multiplicity of infection of at least 10 pfu/cell. Cells were incubated at 37°C for 36-48 hours to allow for total lysis. The lysate was harvested and 0.5% Nonidet P-40 was added followed by shaking at room temperature for 10 minutes to insure complete mixing. Cell debris was removed by centrifugation and the supernatant was incubated, with shaking, overnight in an 0.5 volume of 20% polyethylene glycol/8000/2.5 M NaCl. The adenovirus was pelleted and resuspended in 3-6 ml phosphate-buffered saline (PBS) and centrifuged to remove debris. Cesium chloride was added to the supernatant until 1 ml of solution weighed 1.32-1.34 g. The solution was then subjected to high speed centrifugation for 3 hours at 361,000 g. The white, adenovirus band was recovered. The virus solution was purified over a Pharmacia PD-10 Sephadex column equilibrated with sterile PBS. The absorption of collected fractions was measured at 260 nm and peak fractions were pooled. The final concentration ranged from $1x10^{12}$ to $1x10^{13}$ virions/ml as measured by optical density at 260 nm. A viral disruption assay was done to measure cytopathic effect by titration of virus on 293 cells to look for cell lysis and measure infectivity of virus preps. For *in vivo* use of the virus, a second plaque purification was performed as described above to measure plaque forming units.

Example 10

Hematopoietic Recovery in Myelosuppressed Mice

Thirty five female Balb/c mice, approximately 7 weeks old (Jackson Labs, Bar Harbor, ME) were weighed and

blood was drawn (day -3). On day -1, all mice received myelosuppressive therapy (0.6 mg carboplatin and 350 cGy irradiation). The following day (day 0) the mice were divided into four groups and received 0.1 ml virus ($1x10^{11}$ virus particles) by intravenous tail vein injection. Injection should result in infection of the host's liver and expression of virally delivered gene should commence within 24 hours and continue for 1 to 4 weeks.

Group 1	AdCMV-null (empty virus)	n=10
Group 2	AdCMV-zsig-25 NF	n=10
Group 3	AdCMV-zsig-25 CF	n=10
Group 4	irradiation only	n=5

Blood was drawn on days 6, 11, 15 18, 22 and 25 and body weight taken. Complete blood cell counts were made at each time point. On day 25 red blood cell counts returned to normal ($9x10^6$ cells/µl). The animals were euthanized, and sacrificed by cervical dislocation, and histology was performed on selected organs.

Platelet recovery time was significantly reduced on days 11, 15 and 18 in mice receiving AdCMV-zsig-25 CF. Platelet counts in mice receiving empty virus and those receiving zsig25 NF returned to normal on day 15. Mice receiving AdCMV-zsig-25 CF and those receiving irradiation only returned to normal platelet counts on days 22 and 25 respectively (Figure 7).

Clinical blood chemistry was done at the time of sacrifice and glucose, BUN, creatinine, calcium, phosphorus, total protein, albumin, globulin, albumin/globulin ratio, bilirubin, BBT and cholesterol were all within normal range. Liver enzymes, ALT and AST, were higher in the virus treated mice when compared to the untreated controls. ALT levels were higher in the AdCMV-null and AdCMV-zsig-25CF treated mice when compared to the AdCMV-zsig-25NF treated mice which correlated with the adenovirus dosage given to the mice.

Liver, spleen, kidney, adrenal gland, salivary gland and femur were removed from the treated and untreated mice and subjected to further histopathological analysis. Tissues were scored for degree of liver inflammation, splenic lymphopenia, extramedullary hematopoiesis and bone marrow cellularity. The mean scores were derived by summing the scores for each indication and dividing by the number of animals in that group. A statistically significant difference (by the Kruskal-Wallis and Dunn's Multiple Comparisons Tests) was found between the degree of liver inflammation in the AdCMV-null treated animals and the AdCMV-zsig-25NF treated animals.

Table 5

	Mean Scores			
Treatment	Liver	Lymphopenia	Extra. Hemato.	Bone Marrow
AdCMV-null	1.7	2.0	1.8	3.7
Ad-zsig25NF	0.9	1.4	1.6	3.5
Ad-zsig25CF	1.2	2.3	2.3	3.1
Untreated	0.8	1.3	2.0	3.8

Liver scoring system

Score	
0	Normal
1	Minimal to mild inflammation, no other changes.
2	Minimal inflammation with one or more additional changes[1] .
3	Mild inflammation with one or more additional changes[1].
4	Moderate inflammation with one or more additional changes[1].
5	Severe inflammation with one or more additional changes[1].

[1] Additional changes: cellular pleomorphism, mitotic figures, single hepatocyte necrosis, prominent sinusoidal lining cells.

Splenic lymphopenia and extramedullary hematopoiesis Scoring System

Score	
0	No change
S1	minimal
s2	mild
s3	moderate
s4	severe

Bone marrow cellularity Scoring System

Score	
M1	<25% hematopoietic cells
M2	26-50% hematopoietic cells
M3	51-75% hematopoietic cells
M4	>75% hematopoietic cells

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

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(ii) TITLE OF THE INVENTION: SECRETED F-SPONDIN HOMOLOGS

(iii) NUMBER OF SEQUENCES: 63

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

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(C) REFERENCE/DOCKET NUMBER: 97-07PC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 206-442-6675
(B) TELEFAX: 206-442-6678
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1607 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 40...1032
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GAATTCGGCT CGAGGCGCTC CCGCTGCTCC TGCCGGGTG ATG GAA AAC CCC AGC      54
                                           Met Glu Asn Pro Ser
                                            1               5

CCG GCC GCC GCC CTG GGC AAG GCC CTC TGC GCT CTC CTC CTG GCC ACT    102
Pro Ala Ala Ala Leu Gly Lys Ala Leu Cys Ala Leu Leu Leu Ala Thr
               10                  15                  20

CTC GGC GCC GCC GGC CAG CCT CTT GGG GGA GAG TCC ATC TGT TCC GCC    150
Leu Gly Ala Ala Gly Gln Pro Leu Gly Gly Glu Ser Ile Cys Ser Ala
           25                  30                  35

AGA GCC CCG GCC AAA TAC AGC ATC ACC TTC ACG GGC AAG TGG AGC CAG    198
Arg Ala Pro Ala Lys Tyr Ser Ile Thr Phe Thr Gly Lys Trp Ser Gln
       40                  45                  50

ACG GCC TTC CCC AAG CAG TAC CCC CTG TTC CGC CCC CCT GCG CAG TGG    246
Thr Ala Phe Pro Lys Gln Tyr Pro Leu Phe Arg Pro Pro Ala Gln Trp
   55                  60                  65
```

```
TCT TCG CTG CTG GGG GCC GCG CAT AGC TCC GAC TAC AGC ATG TGG AGG      294
Ser Ser Leu Leu Gly Ala Ala His Ser Ser Asp Tyr Ser Met Trp Arg
70                  75                  80                  85

AAG AAC CAG TAC GTC AGT AAC GGG CTG CGC GAC TTT GCG GAG CGC GGC      342
Lys Asn Gln Tyr Val Ser Asn Gly Leu Arg Asp Phe Ala Glu Arg Gly
                90                  95                  100

GAG GCC TGG GCG CTG ATG AAG GAG ATC GAG GCG GCG GGG GAG GCG CTG      390
Glu Ala Trp Ala Leu Met Lys Glu Ile Glu Ala Ala Gly Glu Ala Leu
            105                 110                 115

CAG AGC GTG CAC GAG GTG TTT TCG GCG CCC GCC GTC CCC AGC GGC ACC      438
Gln Ser Val His Glu Val Phe Ser Ala Pro Ala Val Pro Ser Gly Thr
        120                 125                 130

GGG CAG ACG TCG GCG GAG CTG GAG GTG CAG CGC AGG CAC TCG CTG GTC      486
Gly Gln Thr Ser Ala Glu Leu Glu Val Gln Arg Arg His Ser Leu Val
    135                 140                 145

TCG TTT GTG GTG CGC ATC GTG CCC AGC CCC GAC TGG TTC GTG GGC GTG      534
Ser Phe Val Val Arg Ile Val Pro Ser Pro Asp Trp Phe Val Gly Val
150                 155                 160                 165

GAC AGC CTG GAC CTG TGC GAC GGG GAC CGT TGG CGG GAA CAG GCG GCG      582
Asp Ser Leu Asp Leu Cys Asp Gly Asp Arg Trp Arg Glu Gln Ala Ala
                170                 175                 180

CTG GAC CTG TAC CCC TAC GAC GCC GGG ACG GAC AGC GGC TTC ACC TTC      630
Leu Asp Leu Tyr Pro Tyr Asp Ala Gly Thr Asp Ser Gly Phe Thr Phe
            185                 190                 195

TCC TCC CCC AAC TTC GCC ACC ATC CCG CAG GAC ACG GTG ACC GAG ATA      678
Ser Ser Pro Asn Phe Ala Thr Ile Pro Gln Asp Thr Val Thr Glu Ile
        200                 205                 210

ACG TCC TCC TCT CCC AGC CAC CCG GCC AAC TCC TTC TAC TAC CCG CGG      726
Thr Ser Ser Ser Pro Ser His Pro Ala Asn Ser Phe Tyr Tyr Pro Arg
    215                 220                 225

CTG AAG GCC CTG CCT CCC ATC GCC AGG GTG ACA CTG CTG CGG CTG CGA      774
Leu Lys Ala Leu Pro Pro Ile Ala Arg Val Thr Leu Leu Arg Leu Arg
230                 235                 240                 245
```

```
CAG AGC CCC AGG GCC TTC ATC CCT CCC GCC CCA GTC CTG CCC AGC AGG     822
Gln Ser Pro Arg Ala Phe Ile Pro Pro Ala Pro Val Leu Pro Ser Arg
                250                 255                 260

GAC AAT GAG ATT GTA GAC AGC GCC TCA GTT CCA GAA ACG CCG CTG GAC     870
Asp Asn Glu Ile Val Asp Ser Ala Ser Val Pro Glu Thr Pro Leu Asp
            265                 270                 275

TGC GAG GTC TCC CTG TGG TCG TCC TGG GGA CTG TGC GGA GGC CAC TGT     918
Cys Glu Val Ser Leu Trp Ser Ser Trp Gly Leu Cys Gly Gly His Cys
        280                 285                 290

GGG AGG CTC GGG ACC AAG AGC AGG ACT CGC TAC GTC CGG GTC CAG CCC     966
Gly Arg Leu Gly Thr Lys Ser Arg Thr Arg Tyr Val Arg Val Gln Pro
    295                 300                 305

GCC AAC AAC GGG AGC CCC TGC CCC GAG CTC GAA GAA GAG GCT GAG TGC    1014
Ala Asn Asn Gly Ser Pro Cys Pro Glu Leu Glu Glu Glu Ala Glu Cys
310                 315                 320                 325

GTC CCT GAT AAC TGC GTC TAAGACCAGA GCCCCGCAGC CCCTGGGGCC CCCCGGAG  1070
Val Pro Asp Asn Cys Val
                330

CCATGGGGTG TCGGGGGCTC CTGTGCAGGC TCATGCTGCA GGCGGCCGAG GGCACAGGGG  1130
GTTTCGCGCT GCTCCTGACC GCGGTGAGGC CGCGCCGACC ATCTCTGCAC TGAAGGGCCC  1190
TCTGGTGGCC GGCACGGGCA TTGGGAAACA GCCTCCTCCT TTCCCAACCT TGCTTCTTAG  1250
GGGCCCCCGT GTCCCGTCTG CTCTCAGCCT CCTCCTCCTG CAGGATAAAG TCATCCCCAA  1310
GGCTCCAGCT ACTCTAAATT ATGTCTCCTT ATAAGTTATT GCTGCTCCAG GAGATTGTCC  1370
TTCATCGTCC AGGGGCCTGG CTCCCACGTG GTTGCAGATA CCTCAGACCT GGTGCTCTAG  1430
GCTGTGCTGA GCCCACTCTC CCGAGGGCGC ATCCAAGCGG GGGCCACTTG AGAAGTGAAT  1490
AAATGGGGCG GTTTCGGAAG CGTCAGTGTT TCCATGTTAT GGATCTCTCT GCGTTTGAAT  1550
AAAGACTATC TCTGTTGATC ACAAAAAAAA AAAAAAAAAA AAAAAAAGGG CGGCCGC     1607
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 331 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Asn Pro Ser Pro Ala Ala Ala Leu Gly Lys Ala Leu Cys Ala
 1               5                  10                  15
Leu Leu Leu Ala Thr Leu Gly Ala Ala Gly Gln Pro Leu Gly Gly Glu
            20                  25                  30
Ser Ile Cys Ser Ala Arg Ala Pro Ala Lys Tyr Ser Ile Thr Phe Thr
        35                  40                  45
Gly Lys Trp Ser Gln Thr Ala Phe Pro Lys Gln Tyr Pro Leu Phe Arg
    50                  55                  60
Pro Pro Ala Gln Trp Ser Ser Leu Leu Gly Ala Ala His Ser Ser Asp
65                  70                  75                  80
Tyr Ser Met Trp Arg Lys Asn Gln Tyr Val Ser Asn Gly Leu Arg Asp
                85                  90                  95
Phe Ala Glu Arg Gly Glu Ala Trp Ala Leu Met Lys Glu Ile Glu Ala
            100                 105                 110
Ala Gly Glu Ala Leu Gln Ser Val His Glu Val Phe Ser Ala Pro Ala
        115                 120                 125
Val Pro Ser Gly Thr Gly Gln Thr Ser Ala Glu Leu Glu Val Gln Arg
    130                 135                 140
Arg His Ser Leu Val Ser Phe Val Val Arg Ile Val Pro Ser Pro Asp
145                 150                 155                 160
Trp Phe Val Gly Val Asp Ser Leu Asp Leu Cys Asp Gly Asp Arg Trp
                165                 170                 175
Arg Glu Gln Ala Ala Leu Asp Leu Tyr Pro Tyr Asp Ala Gly Thr Asp
            180                 185                 190
Ser Gly Phe Thr Phe Ser Ser Pro Asn Phe Ala Thr Ile Pro Gln Asp
        195                 200                 205
Thr Val Thr Glu Ile Thr Ser Ser Ser Pro Ser His Pro Ala Asn Ser
    210                 215                 220
Phe Tyr Tyr Pro Arg Leu Lys Ala Leu Pro Pro Ile Ala Arg Val Thr
225                 230                 235                 240
Leu Leu Arg Leu Arg Gln Ser Pro Arg Ala Phe Ile Pro Pro Ala Pro
                245                 250                 255
Val Leu Pro Ser Arg Asp Asn Glu Ile Val Asp Ser Ala Ser Val Pro
            260                 265                 270
Glu Thr Pro Leu Asp Cys Glu Val Ser Leu Trp Ser Ser Trp Gly Leu
        275                 280                 285
Cys Gly Gly His Cys Gly Arg Leu Gly Thr Lys Ser Arg Thr Arg Tyr
    290                 295                 300
Val Arg Val Gln Pro Ala Asn Asn Gly Ser Pro Cys Pro Glu Leu Glu
305                 310                 315                 320
Glu Glu Ala Glu Cys Val Pro Asp Asn Cys Val
                325                 330
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 807 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Arg Leu Ser Pro Ala Pro Leu Arg Leu Ser Arg Gly Pro Ala Leu
 1               5                  10                  15
Leu Ala Leu Ala Leu Pro Leu Ala Ala Ala Leu Ala Phe Ser Asp Glu
            20                  25                  30
Thr Leu Asp Lys Val Ala Lys Ser Glu Gly Tyr Cys Ser Arg Ile Leu
        35                  40                  45
Arg Ala Gln Gly Thr Arg Arg Glu Gly Tyr Thr Glu Phe Ser Leu Arg
    50                  55                  60
Val Glu Gly Asp Pro Asp Phe Tyr Lys Pro Gly Ser Ser Tyr Arg Val
65                  70                  75                  80
Thr Leu Ser Ala Ala Pro Pro Ser Tyr Phe Arg Gly Phe Thr Leu Ile
                85                  90                  95
Ala Leu Lys Glu Asn Arg Glu Gly Asp Lys Glu Glu Asp His Ala Gly
            100                 105                 110
Thr Phe Gln Ile Ile Asp Glu Glu Glu Thr Gln Phe Met Ser Asn Cys
        115                 120                 125
Pro Val Ala Val Thr Glu Ser Thr Pro Arg Arg Arg Thr Arg Ile Gln
    130                 135                 140
Val Phe Trp Ile Ala Pro Pro Thr Gly Thr Gly Cys Val Ile Leu Lys
145                 150                 155                 160
Ala Ser Ile Val Gln Lys Arg Ile Ile Tyr Phe Gln Asp Glu Gly Ser
                165                 170                 175
Leu Thr Lys Lys Leu Cys Glu Gln Asp Pro Thr Leu Asp Gly Val Thr
            180                 185                 190
Asp Arg Pro Ile Leu Asp Cys Cys Ala Cys Gly Thr Ala Lys Tyr Arg
        195                 200                 205
Leu Thr Phe Tyr Gly Asn Trp Ser Glu Lys Thr His Pro Lys Asp Tyr
    210                 215                 220
Pro Arg Arg Ala Asn His Trp Ser Ala Ile Ile Gly Gly Ser His Ser
225                 230                 235                 240
Lys Asn Tyr Val Leu Trp Glu Tyr Gly Gly Tyr Ala Ser Glu Gly Val
                245                 250                 255
```

Lys Gln Val Ala Glu Leu Gly Ser Pro Val Lys Met Glu Glu Glu Ile
260 265 270
Arg Gln Gln Ser Asp Glu Val Leu Thr Val Ile Lys Ala Lys Ala Gln
275 280 285
Trp Pro Ser Trp Gln Pro Val Asn Val Arg Ala Ala Pro Ser Ala Glu
290 295 300
Phe Ser Val Asp Arg Thr Arg His Leu Met Ser Phe Leu Thr Met Met
305 310 315 320
Gly Pro Ser Pro Asp Trp Asn Val Gly Leu Ser Ala Glu Asp Leu Cys
325 330 335
Thr Lys Glu Cys Gly Trp Val Gln Lys Val Val Gln Asp Leu Ile Pro
340 345 350
Trp Asp Ala Gly Thr Asp Ser Gly Val Thr Tyr Glu Ser Pro Asn Lys
355 360 365
Pro Thr Ile Pro Gln Glu Lys Ile Arg Pro Leu Thr Ser Leu Asp His
370 375 380
Pro Gln Ser Pro Phe Tyr Asp Pro Glu Gly Gly Ser Ile Thr Gln Val
385 390 395 400
Ala Arg Val Val Ile Glu Arg Ile Ala Arg Lys Gly Glu Gln Cys Asn
405 410 415
Ile Val Pro Asp Asn Val Asp Asp Ile Val Ala Asp Leu Ala Pro Glu
420 425 430
Glu Lys Asp Glu Asp Asp Thr Pro Glu Thr Cys Ile Tyr Ser Asn Trp
435 440 445
Ser Pro Trp Ser Ala Cys Ser Ser Ser Thr Cys Glu Lys Gly Lys Arg
450 455 460
Met Arg Gln Arg Met Leu Lys Ala Gln Leu Asp Leu Ser Val Pro Cys
465 470 475 480
Pro Asp Thr Gln Asp Phe Gln Pro Cys Met Gly Pro Gly Cys Ser Asp
485 490 495
Glu Asp Gly Ser Thr Cys Thr Met Ser Glu Trp Ile Thr Trp Ser Pro
500 505 510
Cys Ser Val Ser Cys Gly Met Gly Met Arg Ser Arg Glu Arg Tyr Val
515 520 525
Lys Gln Phe Pro Glu Asp Gly Ser Val Cys Met Leu Pro Thr Glu Glu
530 535 540
Thr Glu Lys Cys Thr Val Asn Glu Glu Cys Ser Pro Ser Ser Cys Leu
545 550 555 560
Val Thr Glu Trp Gly Glu Trp Asp Asp Cys Ser Ala Thr Cys Gly Met
565 570 575
Gly Met Lys Lys Arg His Arg Met Val Lys Met Ser Pro Ala Asp Gly
580 585 590
Ser Met Cys Lys Ala Glu Thr Ser Gln Ala Glu Lys Cys Met Met Pro
595 600 605

```
Glu Cys His Thr Ile Pro Cys Leu Leu Ser Pro Trp Ser Glu Trp Ser
    610                 615                 620
Asp Cys Ser Val Thr Cys Gly Lys Gly Met Arg Thr Arg Gln Arg Met
625                 630                 635                 640
Leu Lys Ser Leu Ala Glu Leu Gly Asp Cys Asn Glu Asp Leu Glu Gln
                645                 650                 655
Ala Glu Lys Cys Met Leu Pro Glu Cys Pro Ile Asp Cys Glu Leu Ser
            660                 665                 670
Glu Trp Ser Gln Trp Ser Glu Cys Asn Lys Ser Cys Gly Lys Gly His
        675                 680                 685
Met Ile Arg Thr Arg Thr Ile Gln Met Glu Pro Gln Phe Gly Gly Ala
    690                 695                 700
Pro Cys Pro Glu Thr Val Gln Arg Lys Lys Cys Arg Ala Arg Lys Cys
705                 710                 715                 720
Leu Arg Ser Pro Ser Ile Gln Lys Leu Arg Trp Arg Glu Ala Arg Glu
                725                 730                 735
Ser Arg Arg Ser Glu Gln Leu Arg Glu Glu Ser Asp Gly Glu Gln Phe
            740                 745                 750
Pro Gly Cys Arg Met Arg Pro Trp Thr Ala Trp Ser Glu Cys Thr Lys
        755                 760                 765
Leu Cys Gly Gly Gly Ile Gln Glu Arg Tyr Met Thr Val Lys Lys Arg
    770                 775                 780
Phe Lys Ser Ser Gln Phe Thr Ser Cys Lys Asp Lys Lys Glu Ile Arg
785                 790                 795                 800
Ala Cys Asn Val His Pro Cys
                805
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Leu Cys Ala Leu Leu Leu Ala Thr Leu Gly Ala Ala Gly Gln Pro
 1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC12352

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCTGGCGGAA CAGATGGACT CTCCCCCAAG AGG 33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC12490

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGCTGGTCTC GTTTGTGGTG 20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC12491

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCGGGCGCCG AAAACACCTC 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13387

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CAGCATGTGG AGGAAGAACC 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13388

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAAGCTCGGG ACCAAGAACA 20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13389

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GAGCCTTGGG GATGACTTTA 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13394

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCCGCCTCGA TCTCCTTCAT 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13455

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ACAGCGGCTT CACCTTCTCC 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:

(B) CLONE: ZC13456

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGAACTGAGG CGCTGTCTAC 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13457

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCACGGGCAT TGGGAAACAG 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC13390

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAAGGTTGGG AAAGGAGGAG 20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC447

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TAACAATTTC ACACAGG 17

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC976

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGTTGTAAAA CGACGGCC 18

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCCCCGGCCA AATACAGC 18

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CCAGCAGCGA AGACCACT 18

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCGCGAATTC ATGGAAAACC CCAGC 25

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGCGGGATCC GACGCAGTTA TCAGG 25

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GCGCGGATCC CAGCCTCTTG GGGGA 25

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGCGCTCGAG TTAGACGCAG TTATC 25

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 993 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
ATGGARAAYC CNWSNCCNGC NGCNGCNYTN GGNAARGCNY TNTGYGCNYT NYTNYTNGCN     60
ACNYTNGGNG CNGCNGGNCA RCCNYTNGGN GGNGARWSNA THTGYWSNGC NMGNGCNCCN    120
GCNAARTAYW SNATHACNTT YACNGGNAAR TGGWSNCARA CNGCNTTYCC NAARCARTAY    180
CCNYTNTTYM GNCCNCCNGC NCARTGGWSN WSNYTNYTNG GNGCNGCNCA YWSNWSNGAY    240
TAYWSNATGT GGMGNAARAA YCARTAYGTN WSNAAYGGNY TNMGNGAYTT YGCNGARMGN    300
GGNGARGCNT GGGCNYTNAT GAARGARATH GARGCNGCNG GNGARGCNYT NCARWSNGTN    360
CAYGARGTNT TYWSNGCNCC NGCNGTNCCN WSNGGNACNG GNCARACNWS NGCNGARYTN    420
GARGTNCARM GNMGNCAYWS NYTNGTNWSN TTYGTNGTNM GNATHGTNCC NWSNCCNGAY    480
TGGTTYGTNG GNGTNGAYWS NYTNGAYYTN TGYGAYGGNG AYMGNTGGMG NGARCARGCN    540
GCNYTNGAYY TNTAYCCNTA YGAYGCNGGN ACNGAYWSNG GNTTYACNTT YWSNWSNCCN    600
AAYTTYGCNA CNATHCCNCA RGAYACNGTN ACNGARATHA CNWSNWSNWS NCCNWSNCAY    660
CCNGCNAAYW SNTTYTAYTA YCCNMGNYTN AARGCNYTNC CNCCNATHGC NMGNGTNACN    720
YTNYTNMGNY TNMGNCARWS NCCNMGNGCN TTYATHCCNC CNGCNCCNGT NYTNCCNWSN    780
MGNGAYAAYG ARATHGTNGA YWSNGCNWSN GTNCCNGARA CNCCNYTNGA YTGYGARGTN    840
WSNYTNTGGW SNWSNTGGGG NYTNTGYGGN GGNCAYTGYG GNMGNYTNGG NACNAARWSN    900
MGNACNMGNT AYGTNMGNGT NCARCCNGCN AAYAAYGGNW SNCCNTGYCC NGARYTNGAR    960
GARGARGCNG ARTGYGTNCC NGAYAAYTGY GTN                                 993
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 623 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Met Asn Arg Leu Arg Val Ala Arg Leu Thr Pro Leu Glu Leu Leu Leu
 1               5                  10                  15
Ser Leu Met Ser Leu Leu Leu Gly Thr Arg Pro His Gly Ser Pro Gly
            20                  25                  30
Pro Leu Gln Cys Tyr Ser Val Gly Pro Leu Gly Ile Leu Asn Cys Ser
        35                  40                  45
Trp Glu Pro Leu Gly Asp Leu Glu Thr Pro Pro Val Leu Tyr His Gln
    50                  55                  60
Ser Gln Lys Tyr His Pro Asn Arg Val Trp Glu Val Lys Val Pro Ser
65                  70                  75                  80
Lys Gln Ser Trp Val Thr Ile Pro Arg Glu Gln Phe Thr Met Ala Asp
                85                  90                  95
```

Lys Leu Leu Ile Trp Gly Thr Gln Lys Gly Arg Pro Leu Trp Ser Ser
100 105 110
Val Ser Val Asn Leu Glu Thr Gln Met Lys Pro Asp Thr Pro Gln Ile
115 120 125
Phe Ser Gln Val Asp Ile Ser Glu Glu Ala Thr Leu Glu Ala Thr Val
130 135 140
Gln Trp Ala Pro Pro Val Trp Pro Pro Gln Lys Ala Leu Thr Cys Gln
145 150 155 160
Phe Arg Tyr Lys Glu Cys Gln Ala Glu Ala Trp Thr Arg Leu Glu Pro
165 170 175
Gln Leu Lys Thr Asp Gly Leu Thr Pro Val Glu Met Gln Asn Leu Glu
180 185 190
Pro Gly Thr Cys Tyr Gln Val Ser Gly Arg Cys Gln Val Glu Asn Gly
195 200 205
Tyr Pro Trp Gly Glu Trp Ser Ser Pro Leu Ser Phe Gln Thr Pro Phe
210 215 220
Leu Asp Pro Glu Asp Val Trp Val Ser Gly Thr Val Cys Glu Thr Ser
225 230 235 240
Gly Lys Arg Ala Ala Leu Leu Val Trp Lys Asp Pro Arg Pro Cys Val
245 250 255
Gln Val Thr Tyr Thr Val Trp Phe Gly Ala Gly Asp Ile Thr Thr Thr
260 265 270
Gln Glu Glu Val Pro Cys Cys Lys Ser Pro Val Pro Ala Trp Met Glu
275 280 285
Trp Ala Val Val Ser Pro Gly Asn Ser Thr Ser Trp Val Pro Pro Thr
290 295 300
Asn Leu Ser Leu Val Cys Leu Ala Pro Glu Ser Ala Pro Cys Asp Val
305 310 315 320
Gly Val Ser Ser Ala Asp Gly Ser Pro Gly Ile Lys Val Thr Trp Lys
325 330 335
Gln Gly Thr Arg Lys Pro Leu Glu Tyr Val Val Asp Trp Ala Gln Asp
340 345 350
Gly Asp Ser Leu Asp Lys Leu Asn Trp Thr Arg Leu Pro Pro Gly Asn
355 360 365
Leu Ser Thr Leu Leu Pro Gly Glu Phe Lys Gly Gly Val Pro Tyr Arg
370 375 380
Ile Thr Val Thr Ala Val Tyr Ser Gly Gly Leu Ala Ala Ala Pro Ser
385 390 395 400
Val Trp Gly Phe Arg Glu Glu Leu Val Pro Leu Ala Gly Pro Ala Val
405 410 415
Trp Arg Leu Pro Asp Asp Pro Pro Gly Thr Pro Val Val Ala Trp Gly
420 425 430
Glu Val Pro Arg His Gln Leu Arg Gly Gln Ala Thr His Tyr Thr Phe
435 440 445

```
Cys Ile Gln Ser Arg Gly Leu Ser Thr Val Cys Arg Asn Val Ser Ser
    450                 455                 460
Gln Thr Gln Thr Ala Thr Leu Pro Asn Leu His Ser Gly Ser Phe Lys
465                 470                 475                 480
Leu Trp Val Thr Val Ser Thr Val Ala Gly Gln Gly Pro Pro Gly Pro
                485                 490                 495
Asp Leu Ser Leu His Leu Pro Asp Asn Arg Ile Arg Trp Lys Ala Leu
            500                 505                 510
Pro Trp Phe Leu Ser Leu Trp Gly Leu Leu Leu Met Gly Cys Gly Leu
        515                 520                 525
Ser Leu Ala Ser Thr Arg Cys Leu Gln Ala Arg Cys Leu His Trp Arg
    530                 535                 540
His Lys Leu Leu Pro Gln Trp Ile Trp Glu Arg Val Pro Asp Pro Ala
545                 550                 555                 560
Asn Ser Asn Ser Gly Gln Pro Tyr Ile Lys Glu Val Ser Leu Pro Gln
                565                 570                 575
Pro Pro Lys Asp Gly Pro Ile Leu Glu Val Glu Glu Val Glu Leu Gln
            580                 585                 590
Pro Val Val Glu Ser Pro Lys Ala Ser Ala Pro Ile Tyr Ser Gly Tyr
        595                 600                 605
Glu Lys His Phe Leu Pro Thr Pro Glu Glu Leu Gly Leu Leu Val
    610                 615                 620
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 633 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Met Pro Ser Trp Ala Leu Phe Met Val Thr Ser Cys Leu Leu Leu Ala
 1               5                  10                  15
Leu Pro Asn Gln Ala Gln Val Thr Ser Gln Asp Val Phe Leu Leu Ala
            20                  25                  30
Leu Gly Thr Glu Pro Leu Asn Cys Phe Ser Gln Thr Phe Glu Asp Leu
        35                  40                  45
Thr Cys Phe Trp Asp Glu Glu Glu Ala Ala Pro Ser Gly Thr Tyr Gln
    50                  55                  60
Leu Leu Tyr Ala Tyr Arg Gly Glu Lys Pro Arg Ala Cys Pro Leu Tyr
65                  70                  75                  80
```

```
Ser Gln Ser Val Pro Thr Phe Gly Thr Arg Tyr Val Cys Gln Phe Pro
                85                  90                  95
Ala Gln Asp Glu Val Arg Leu Phe Phe Pro Leu His Leu Trp Val Lys
            100                 105                 110
Asn Val Ser Leu Asn Gln Thr Leu Ile Gln Arg Val Leu Phe Val Asp
        115                 120                 125
Ser Val Gly Leu Pro Ala Pro Pro Arg Val Ile Lys Ala Arg Gly Gly
    130                 135                 140
Ser Gln Pro Gly Glu Leu Gln Ile His Trp Glu Ala Pro Ala Pro Glu
145                 150                 155                 160
Ile Ser Asp Phe Leu Arg His Glu Leu Arg Tyr Gly Pro Thr Asp Ser
                165                 170                 175
Ser Asn Ala Thr Ala Pro Ser Val Ile Gln Leu Leu Ser Thr Glu Thr
            180                 185                 190
Cys Cys Pro Thr Leu Trp Met Pro Asn Pro Val Pro Val Leu Asp Gln
        195                 200                 205
Pro Pro Cys Val His Pro Thr Ala Ser Gln Pro His Gly Pro Val Arg
    210                 215                 220
Thr Ser Pro Ala Gly Glu Ala Pro Phe Leu Thr Val Lys Gly Gly Ser
225                 230                 235                 240
Cys Leu Val Ser Gly Leu Gln Ala Gly Lys Ser Tyr Trp Leu Gln Leu
                245                 250                 255
Arg Ser Gln Pro Asp Gly Val Ser Leu Arg Gly Ser Trp Gly Pro Trp
            260                 265                 270
Ser Phe Pro Val Thr Val Asp Leu Pro Gly Asp Ala Val Thr Ile Gly
        275                 280                 285
Leu Gln Cys Phe Thr Leu Asp Leu Lys Met Val Thr Cys Gln Trp Gln
    290                 295                 300
Gln Gln Asp Arg Thr Ser Ser Gln Gly Phe Phe Arg His Ser Arg Thr
305                 310                 315                 320
Arg Cys Cys Pro Thr Asp Arg Asp Pro Thr Trp Glu Lys Cys Glu Glu
                325                 330                 335
Glu Glu Pro Arg Pro Gly Ser Gln Pro Ala Leu Val Ser Arg Cys His
            340                 345                 350
Phe Lys Ser Arg Asn Asp Ser Val Ile His Ile Leu Val Glu Val Thr
        355                 360                 365
Thr Ala Gln Gly Ala Val His Ser Tyr Leu Gly Ser Pro Phe Trp Ile
    370                 375                 380
His Gln Ala Val Leu Leu Pro Thr Pro Ser Leu His Trp Arg Glu Val
385                 390                 395                 400
Ser Ser Gly Arg Leu Glu Leu Glu Trp Gln His Gln Ser Ser Trp Ala
                405                 410                 415
Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly Glu Gly Arg Glu
            420                 425                 430
```

```
Asp Trp Lys Val Leu Glu Pro Ser Leu Gly Ala Arg Gly Gly Thr Leu
        435                 440                 445
Glu Leu Arg Pro Arg Ala Arg Tyr Ser Leu Gln Leu Arg Ala Arg Leu
    450                 455                 460
Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ala Trp Ser Pro Pro Ala
465                 470                 475                 480
Arg Val Ser Thr Gly Ser Glu Thr Ala Trp Ile Thr Leu Val Thr Ala
                485                 490                 495
Leu Leu Leu Val Leu Ser Leu Ser Ala Leu Leu Gly Leu Leu Leu Leu
            500                 505                 510
Lys Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu Trp
        515                 520                 525
Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg Asp
    530                 535                 540
Thr Ala Ala Leu Ser Pro Ser Lys Ala Thr Val Thr Asp Ser Cys Glu
545                 550                 555                 560
Glu Val Glu Pro Ser Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu Ser
                565                 570                 575
Thr Pro Leu Pro Leu Cys Pro Ser Gln Pro Gln Met Asp Tyr Arg Gly
            580                 585                 590
Leu Gln Pro Cys Leu Arg Thr Met Pro Leu Ser Val Cys Pro Pro Met
        595                 600                 605
Ala Glu Thr Gly Ser Cys Cys Thr Thr His Ile Ala Asn His Ser Tyr
    610                 615                 620
Leu Pro Leu Ser Tyr Trp Gln Gln Pro
625                 630
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CACCAAGGTG ATCCACCTGA TCCTATTATC TGG 33

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AACCTCAGCA CATTGTTACC AGGG 24

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Trp Ile Thr Leu Val Thr Ala
1 5

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CCGCTCGAGT CAGGGCTGCT GCCAATAGCT TAGTGG 36

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AATAGGATCA GGTGGATCAC CTTGGTGACT GCT 33

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Pro Asp Asn Arg Ile Arg
1 5

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Pro Asp Asn Arg Ile Arg Trp Ile Thr Leu Val Thr Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TGGGAGGAAT CAGGGTAG 18

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

AGGCAAAGTC ACATCAGG 18

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 376 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ACATCAGAAG ATAATGAGCA ATAGGATTAA TTCTGGGAGG AATCAGGGTA GTAATAGGAC 60
AATCTTAGCC CCCTGATGTG ACTTTGCCTA CCACGAGTCA ACCCTGAAGA ATTCGACTCA 120
AACCCTAGAA TCATTAGCCA CCAGCACAGG TCAGGCATTA AAAGGGAATA CANGAATCCC 180
TAGACTGTAT GGGCAAATAT AATCCTTGAT AACAGACTAG TGTTGGGTTA TTTAACGAGC 240
TGAACAAGGT GGAGTCTNTA CGGNCTATTT ACCAAACCTG CTGCACCNAT GTTTACCACT 300
TTGGGCCATA ATNATTTTTA CCCTTCCAAA GGGTTTTTGG GGCAGTTCCC NGGGGTNCAT 360
TGGGGNTTAC CCCGGG 376

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 541 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ATTCGGATCC AACGAACTAG TGAGAAGGTT GAGCAGAGAA CCCCTGCAGC AGGAAAACCT 60
CTTGCTTCCT TGTCTCAAGC CCCTTCTGGC ACTTTCTGGG ACCAGGCACT GAGGCAGAAG 120
ACAGGAAGAG CTGGGGCCTA TCTGCTGGCC AGAGGACTAA CCTTGGGCAC ACATAGGATC 180
TGGGAGCTCT GGGACCAATC CTCCAGCTGA CAAGCCCAGC AGCCTGCCTC TGACGCTTTT 240
GCCAGGTGAT GGAAAACGTG AGTCTTGCCC TGGGAGAGCT CTTTGGGTCT TCCTCCTGGC 300
CATGATAGGC TCCACCACGA GCCAGCCGCT GGGGGGAGAA TCGGTTTGTA CAGCCCGGGC 360
TCTGGCTAGA TACAGCATCA CTTTCATTGG CAAGTGGAGC CAGACAGCAT TTCCCAAGCA 420
GTACCCCCTG TTCCGGCCCC CTGCACAGTG GTCCTCTCTG CTGGGGGCAG CTCACAGCTC 480
TGACTACAGC ATGTGGCGGA AGAATGAGTA TGTCAGCAAT GGGCTGAGGG ACTTTGCTGA 540
G 541

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 470 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

AGGTTGAGCA GAGAACCCCT GCAGCAGGAA AACCTCTTGC TTCCTTGTCT CAAGCCCCTT 60
CTGGCACTTT CTGGGACCAG GCACTGAGGC AGAAGACAGG AAGAGCTGGG GCCTATCTGC 120
TGGCCAGAGC GACTAACCTT GGGCACACAT AGGATCTGGG AGCTCTGGGA CCAATCCTCC 180
AGCTGACAAG CACAGCAGCC TGCCTCTGAC GCTTTTGCCA GGTGATGGAA AACGTGAGTC 240
TTGCCCTGGG CAGAGCTCTT TGGGTCTTCC TCCTGGCCAT GATAGGCTCC ACCACGAGCC 300
AGCCGCTGGG GGGAGAATCG GTTTGTACAG CCCGGCCTCT GGCTAGATAC AGCATCACTT 360
TCATTGGCAA GTGGAGCCAG ACAGCATTTC CCAAGCAGTA CCCCCTGTTC CGGCCCCCTG 420
CACAGTGGTC CTCTCTGCTG GGGGCAGCTC ACAGCTCTGA CTACAGCGTN 470

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 553 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TTCGGATCCT TGCTCTTGCT TCCTTGTCTC AAGCCCCTTC TGGCACTTTC TGGGACCAGG 60
CACTGAGGCA GAAGACAGGA AGAGCTGGGG CCTATCTGCT GGCCAGACGA CTAACCTTGG 120
GCACACATAG GATCTGGGAG CTCTGGGACC AATCCTCCAG CTGACAAGCC AGCAGCCTGC 180
CTCTGACGCT TTTGCCAGGT GATGGAAAAC GTGAGTCTTG CCCTGGGAGA GCTCTTTGGG 240
TCTTCCTCCT GGCCATGATA GGCTCCACCA CGAGCCAGCC GCTGGGGGGA GAATCGGTTT 300
GTACAGCCCG GCCTCTGGTA GATACAGCAT CACTTTCATT GGCAAGTGGA GCCAGACAGC 360
ATTTCCCAAG CAGTACCCCC TGTTCCGGCC CCCTGCACAG TGGTCCTCTC TGCTGGGGGC 420
AGCTCACAGC TCTGACTACA GCATGTGGCG GAAGAATGAG TATGTCAGCA ATGGGCTGAG 480
GGACTTTGCT GAGCGTGGTG AGGACTGGGC ACTGATGAAG GAGATCGAAG CTGCAGGAGN 540
AGAACTTCAG AGT 553

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 292 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CTCAAGACCC TTCTGGCACT TTCTGGGACC AGGCACTGAG GCAGAAGACA GGAAGAGCTG 60
GGGCCTATCT GCTGGCCAGA GGACGTAACC TTGGGCACAC ATAGGATCTC GGAGCTCTGG 120
GACCAATCCT CCAGCTGACA AGCCCAGCAG CCTGCCTCTG ACGCTTTTGC CAGGTGATGG 180
AAAACGTGAG TCTTGCCCTG GGCAGAGCTC TTTGGGTCTT CCTCCTGGCC ATGATAGGCT 240
CCACCACGAG CCAGCCGCTG GGGGGAGAAT CGGTTTGTAC AGCCCGGCCT CT 292

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 432 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CATCGCCCAG CCACCCAGCA AATTCATTCT ACTACCCACG CCTCAAGTCC TTACCTCCCA 60
TCGCCAAAGT GACCTTCGTG CGGCTACAGC AGAGTCCCAG GGCCTTTGCC CACCTTCCCT 120
GGACCTGGCC AGCCGAGGCA ACGAAATCGT TGACAGCCTT TCAGTTCCAG AGACACCGCT 180
GGACTGTGAG GTTTCCCTGT GGTCATCCTG GGGACTGTGT GGAGGACCAT GTGGAAAGTT 240
GGGAGCCAAG AGCAGAACTC GCTATGTCCG TGTTCAGCCT GCTAACAATG GGACTCCCTG 300
TCCCGAGCTT GAAGAAGAGG CCGAGTGTGC CCCAGATAAC TGCGTCTAAA CCCAGACTCC 360
AGTAGCAAGC AGCTCTTAGG ATCGCTCAAG GCTGGGACTC AGATCTGGGC CACAAGGTGT 420
TTCCACAGGA GT 432

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GNGCNCCNGC NAARTAY 17

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GNGSNMCNGC NAARTAY 17

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CNCSNKGNCG NTTYART 17

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CANTTYCANG GNAARTG 17

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CANTTYWMNG GNAANTG 17

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

TGNAARWKNC CNTTNAC 17

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TTYCCNAARC ARTAYCC 17

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

YWYCCNAARS ANTAYCC 17

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

RWRGGNTTYS TNARTGG 17

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

CCNGAYTTGT TYGTNGG 17

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

CCNDAYTGGW WYGTNGG 17

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GGNCTRACCW WRCANCC 17

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CCNTAYGAYG CNGGNAC 17

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CCNTRBGAYG CNGGNAC 17

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GGNAYVCRTC GNCCNTG 17

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

SCNCANATHC CNCARGA 17

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

SCNCANATHC CNCARGA 17

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGNAYVCRTC GNCCNTG 17

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GGAGGTCTAT ATAAGCAGAG C 21

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GTGAAATTTG TGATGCTATT GC 22

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

TTATGTTTCA GGTTCAGGGG 20

CLAIMS

What is claimed is:

1. An isolated polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 27-277 of SEQ ID NO:2.

2. An isolated polypeptide according to claim 1, wherein said polypeptide that is at least 90% identical in amino acid sequence to residues 27-277 of SEQ ID NO:2.

3. An isolated polypeptide according to claim 1, wherein said polypeptide comprises residues 1-277 of SEQ ID NO:2.

4. An isolated polypeptide according to claim 1, wherein said polypeptide further comprises one thrombospondin type 1 domain positioned carboxy terminal to said polypeptide.

5. An isolated polypeptide according to claim 4, wherein said thrombospondin type 1 domain comprises residues 278-330 of SEQ ID NO:2.

6. An isolated polypeptide according to claim 1, wherein said polypeptide comprises residues 27-331 of SEQ ID NO:2.

7. An isolated polypeptide according to claim 1, wherein said polypeptide comprises residues 1-331 of SEQ ID NO:2.

8. An isolated polypeptide according to claim 1, wherein said polypeptide is from 251 to 331 amino acid residues in length.

9. An isolated polypeptide according to claim 1, covalently linked amino terminally or carboxy terminally to a

moiety selected from the group consisting of affinity tags, toxins, radionucleotides, enzymes and fluorophores.

10. An isolated polypeptide according to claim 9, wherein said moiety is an affinity tag selected from the group consisting of polyhistidine, SEQ ID NO:20, Glu-Glu, glutathione S transferase and an immunoglobulin heavy chain constant region.

11. An isolated polypeptide according to claim 10 further comprising a proteolytic cleavage site between said sequence of amino acid residues and said affinity tag.

12. A fusion protein consisting essentially of a first portion and a second portion joined by a peptide bond, said first portion comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 27-277 of SEQ ID NO:2; and said second portion consisting essentially of 2 to 8 thrombospondin type 1 domains.

13. A fusion protein comprising a secretory signal sequence having the amino acid sequence of amino acid residues 1-27 of SEQ ID NO:2, wherein said secretory signal sequence is operably linked to an additional polypeptide.

14. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 27-227 of SEQ ID NO:2; and

a transcription terminator.

15. An expression vector according to claim 14, wherein said DNA segment encodes a polypeptide that is at

least 90% identical in amino acid sequence to residues 27-227 of SEQ ID NO:2.

16. An expression vector according to claim 14, wherein said DNA segment encodes a polypeptide comprising residues 1-227 of SEQ ID NO:2.

17. An expression vector according to claim 14, wherein said DNA segment encodes a polypeptide further comprising a thrombospondin type 1 domain positioned carboxy terminal to said polypeptide.

18. An expression vector according to claim 14, wherein said thrombospondin type 1 domain comprises residues 278-330 of SEQ ID NO:2.

19. An expression vector according to claim 14, wherein said DNA segment encodes a polypeptide comprising residues 27-331 of SEQ ID NO:2.

20. An expression vector according to claim 14, wherein said DNA segment encodes a polypeptide comprising residues 1-331 of SEQ ID NO:2.

21. An expression vector according to claim 14, wherein said DNA segment encodes a polypeptide of from 251 to 331 amino acid residues in length.

22. An expression vector according to claim 14, wherein said DNA segment encodes a polypeptide covalently linked amino terminally or carboxy terminally to an affinity tag selected from the group consisting of polyhistidine, SEQ ID NO:20, Glu-Glu, glutathione S transferase and an immunoglobulin heavy chain constant region.

23. An expression vector according to claim 14 wherein said DNA segment further encodes a secretory signal sequence operably linked to said polypeptide.

24. An expression vector according the claim 23, wherein said secretory signal sequence comprises residues 1-26 of SEQ ID NO:2.

25. A cultured cell into which has been introduced an expression vector according to claim 14, wherein said cell expresses said polypeptide encoded by said DNA segment.

26. A method of producing a polypeptide comprising:

culturing a cell into which has been introduced an expression vector according to claim 14 whereby said cell expresses said polypeptide encoded by said DNA segment; and

recovering said expressed polypeptide.

27. A pharmaceutical composition comprising a polypeptide according to claim 1 in combination with a pharmaceutically acceptable vehicle.

28. An antibody that specifically binds to an epitope of a polypeptide according to claim 1.

29. A binding protein that specifically binds to an epitope of a polypeptide according to claim 1.

30. An isolated polynucleotide encoding a polypeptide comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 27-227 of SEQ ID NO:2.

31. An isolated polynucleotide according to claim 30, wherein said polypeptide is at least 90% identical in amino acid sequence to residues 27-227 of SEQ ID NO:2.

32. An isolated polynucleotide according to claim 30, wherein said polypeptide comprises residues 1-227 of SEQ ID NO:2.

33. An isolated polynucleotide according to claim 30, wherein said polypeptide further comprises a thrombospondin type 1 domain carboxy terminal to said polypeptide.

34. An isolated polynucleotide according to claim 30, wherein said thrombospondin type 1 domain comprises residues 278-330 of SEQ ID NO:2.

35. An isolated polynucleotide according to claim 30, wherein said polypeptide comprises residues 27-331 of SEQ ID NO:2.

36. An isolated polynucleotide according to claim 30, wherein said polypeptide comprises residues 1-331 of SEQ ID NO:2.

37. An isolated polynucleotide according to claim 30, wherein said polynucleotide is from 830 to 1032 nucleotides in length.

38. An isolated polynucleotide according to claim 30 comprising nucleotide 1 to nucleotide 993 of SEQ ID NO:25.

39. An isolated polynucleotide according to claim 30, wherein said polynucleotide is DNA.

40. An isolated polynucleotide selected from the group consisting of,

a) a sequence of nucleotides from nucleotide 118 to nucleotide 870 of SEQ ID NO:1;

b) a sequence of nucleotides from nucleotide 118 to nucleotide 1032 of SEQ ID NO:2;

c) a sequence of nucleotides from nucleotide 40 to nucleotide 870 of SEQ ID NO:2;

d) a sequence of nucleotides from nucleotide 40 to nucleotide 1032 of SEQ ID NO:2;

e) species orthologs of a), b), c) or d);

f) allelic variants of a), b), c), d) or e); and

g) nucleotide sequences complementary to a), b), c), d), e) or f).

41. An isolated polynucleotide encoding a fusion protein consisting essentially of a first portion and a second portion joined by a peptide bond, said first portion comprising a sequence of amino acid residues that is at least 80% identical in amino acid sequence to residues 27-277 of SEQ ID NO:2; and said second portion consisting essentially of 2 to 8 thrombospondin type 1 domains.

42. An isolated polypeptide encoding a fusion protein comprising a secretory signal sequence having the amino acid sequence of amino acid residues 1-27 of SEQ ID NO:2, wherein said secretory signal sequence is operably linked to an additional polypeptide.

43. An oligonucleotide probe or primer comprising at least 14 contiguous nucleotides of a polynucleotide of SEQ ID NO:25 or a sequence complementary to SEQ ID NO:25.

44. A method for detecting a genetic abnormality in a patient, comprising:

obtaining a genetic sample from a patient;

incubating the genetic sample with a polynucleotide comprising at least 14 contiguous nucleotides of SEQ ID NO:1 or the complement of SEQ ID NO:1, under conditions wherein said polynucleotide will hybridize to complementary polynucleotide sequence, to produce a first reaction product;

comparing said first reaction product to a control reaction product, wherein a difference between said first reaction product and said control reaction product is indicative of a genetic abnormality in the patient.

```
FSPO_R MRLSPAPLRLSRGPALLALALPLAAALAFSDETLDKVAKSEGYCSRILRAQGTRREGYTE; 60

FSPO_R FSLRVEGDPDFYKPGSSYRVTLSAAPPSYFRGFTLIALKENREGDKEEDHAGTFQIIDEE; 120

FSPO_R ETQFMSNCPVAVTESTPRRRTRIQVFWIAPPTGTGCVILKASIVQKRIIYFQDEGSLTKK; 180
zsig25                                                  MENPSPAAALGKA; 13

FSPO_R LCEQDPTLDGVTDRPILD---CCACGTAKYRLTFYGNWSEKTHPKDYP--RRANHWSAII; 235
zsig25 LCALLLATLGAAGQPLGGESICSARAPAKYSITFTGKWSQTAFPKQYPLFRPPAQWSSLL; 73

FSPO_R GGSHSKNYVLWEYGGYASEGVKQVAELGSPVKMEEEIRQQSDEVLTVIKAKAQWPSWQPV; 295
zsig25 GAAHSSDYSMWRKNQYVSNGLRDFAERGEAWALMKEIEAAGEALQSV---HEVFSAPAVP; 130

FSPO_R NVRAAPSAEFSVDRTRHLMSFLTMMGPSPDWNVGLSAEDLCTKECGWVQKVVQDLIPWDA; 355
zsig25 SGTGQTSAELEVQRRHSLVSFVVRIVPSPDWFVGVDSLDLCDGD-RWREQAALDLYPYDA; 189

FSPO_R GTDSGVTYESPNKPTIPQEKIRPLT--SLDHPQSPFYDPEGGSITQVARVVIERIARKGE; 413
zsig25 GTDSGFTFSSPNFATIPQDTVTEITSSSPSHPANSFYYPRLKALPPIARVTLLRL-RQSP; 248

FSPO_R QCNIVPDNVDDIVADLAPEEKDEDDTPETCIYSNWSPWSACSSSTCEKGKRMRQRMLKAQ; 473
zsig25 RAFIPPAPVLPSRDNEIVDSASVPETPLDCEVSLWSSWGLCGGHCGRLGTKSRTRYVRVQ; 308

FSPO_R -LDLSVPCPDTQDFQPCMGPGCSDEDGSTCTMSEWITWSPCSVSCGMGMRSRERYVKQFP; 532
zsig25 PANNGSPCPELEEEAECVPDNCV.                                    ; 331

FSPO_R EDGSVCMLPTEETEKCTVNEECSPSSCLVTEWGEWDDCSATCGMGMKKRHRMVKMSPADG; 592

FSPO_R SMCKAETSQAEKCMMPECHTIPCLLSPWSEWSDCSVTCGKGMRTRQRMLKSLAELGDCNE; 652

FSPO_R DLEQAEKCMLPECPIDCELSEWSQWSECNKSCGKGHMIRTRTIQMEPQFGGAPCPETVQR; 712

FSPO_R KKCRARKCLRSPSIQKLRWREARESRRSEQLREESDGEQFPGCRMRPWTAWSECTKLCGG; 772

FSPO_R GIQERYMTVKKRFKSSQFTSCKDKKEIRACNVHPC                        ; 807
```

Fig. 1

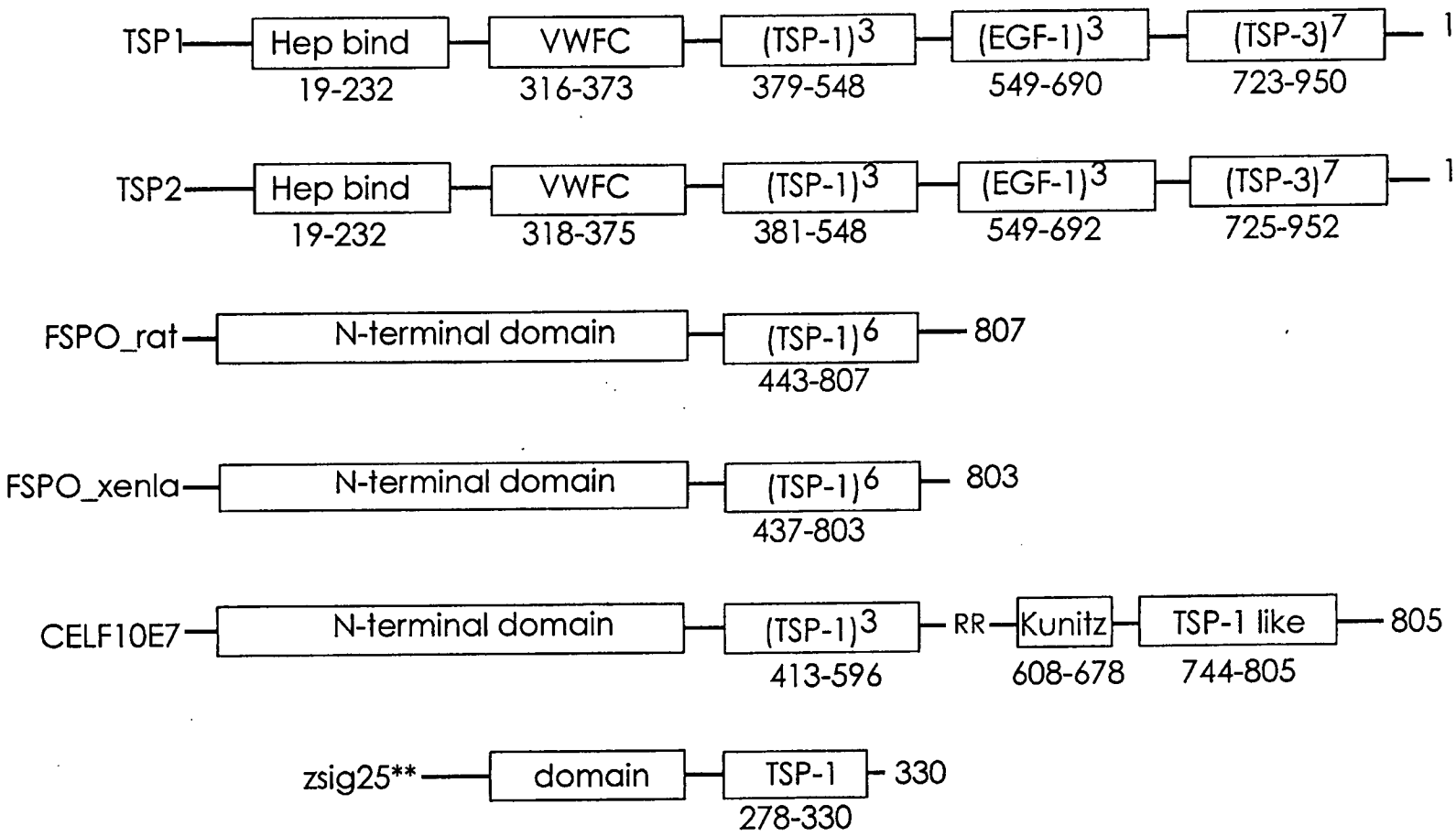

Fig. 2

BaF3 pZR103#2 Cells with zsig25NF (A080F) Dose Response

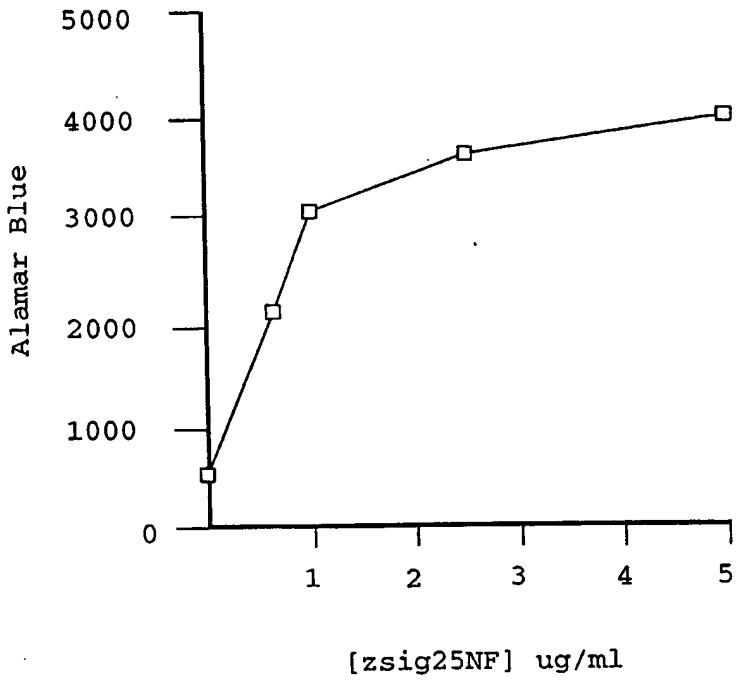

Fig. 3

DA-1 Cells with zsig25NF (A080F)Dose Response

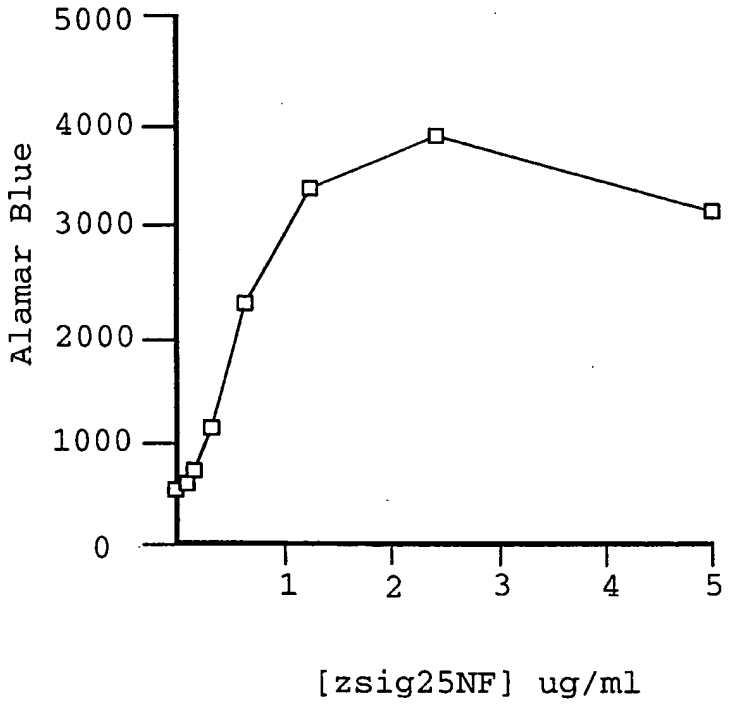

Fig. 4

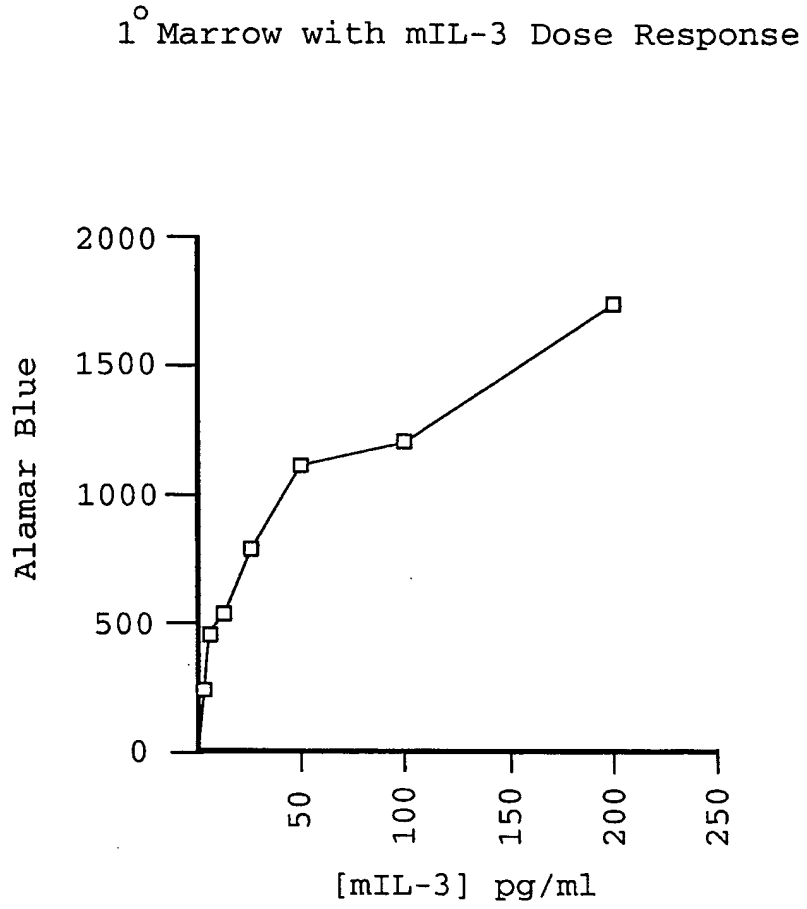
1° Marrow with mIL-3 Dose Response
Alamar Blue
2000
1500
1000
500
0
50
100
150
200
250
[mIL-3] pg/ml

Fig. 5

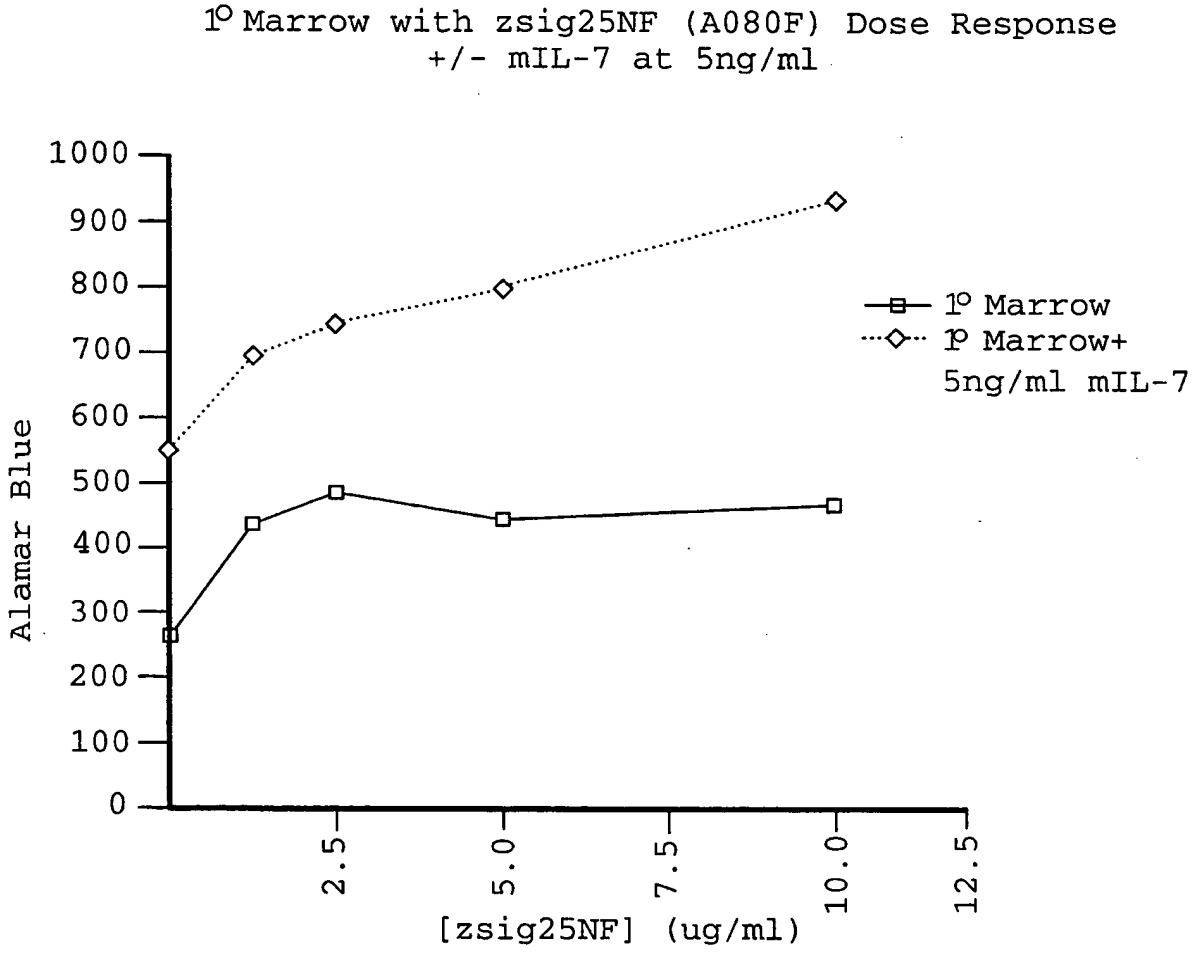
1° Marrow with zsig25NF (A080F) Dose Response
+/- mIL-7 at 5ng/ml
Alamar Blue
1000
900
800
700
600
500
400
300
200
100
0
2.5
5.0
7.5
10.0
12.5
[zsig25NF] (ug/ml)
1° Marrow
1° Marrow+
5ng/ml mIL-7

Fig. 6

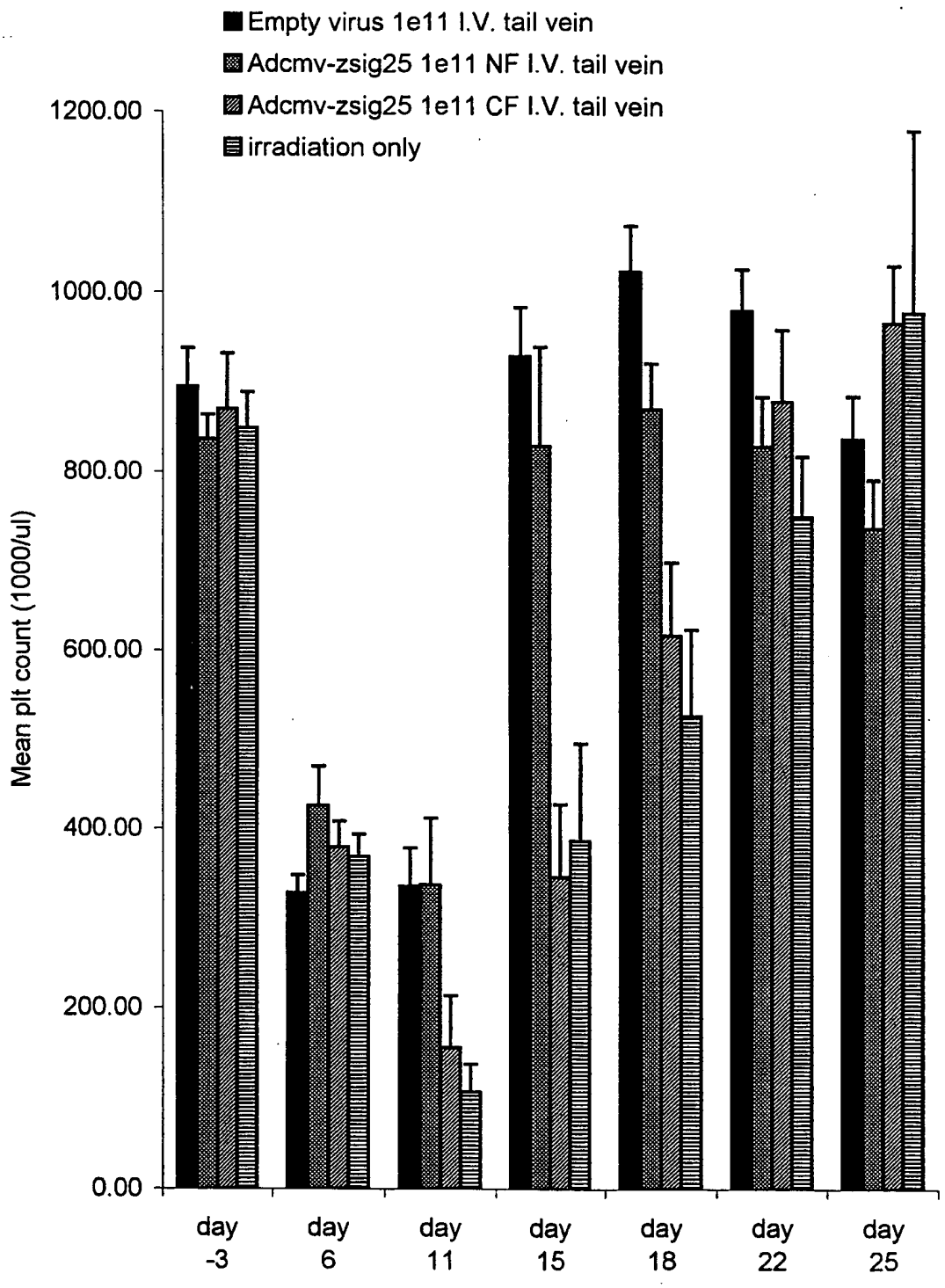
Empty virus 1e11 I.V. tail vein
Adcmv-zsig25 1e11 NF I.V. tail vein
Adcmv-zsig25 1e11 CF I.V. tail vein
irradiation only
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
Mean plt count (1000/ul)
day -3
day 6
day 11
day 15
day 18
day 22
day 25

Figure 7

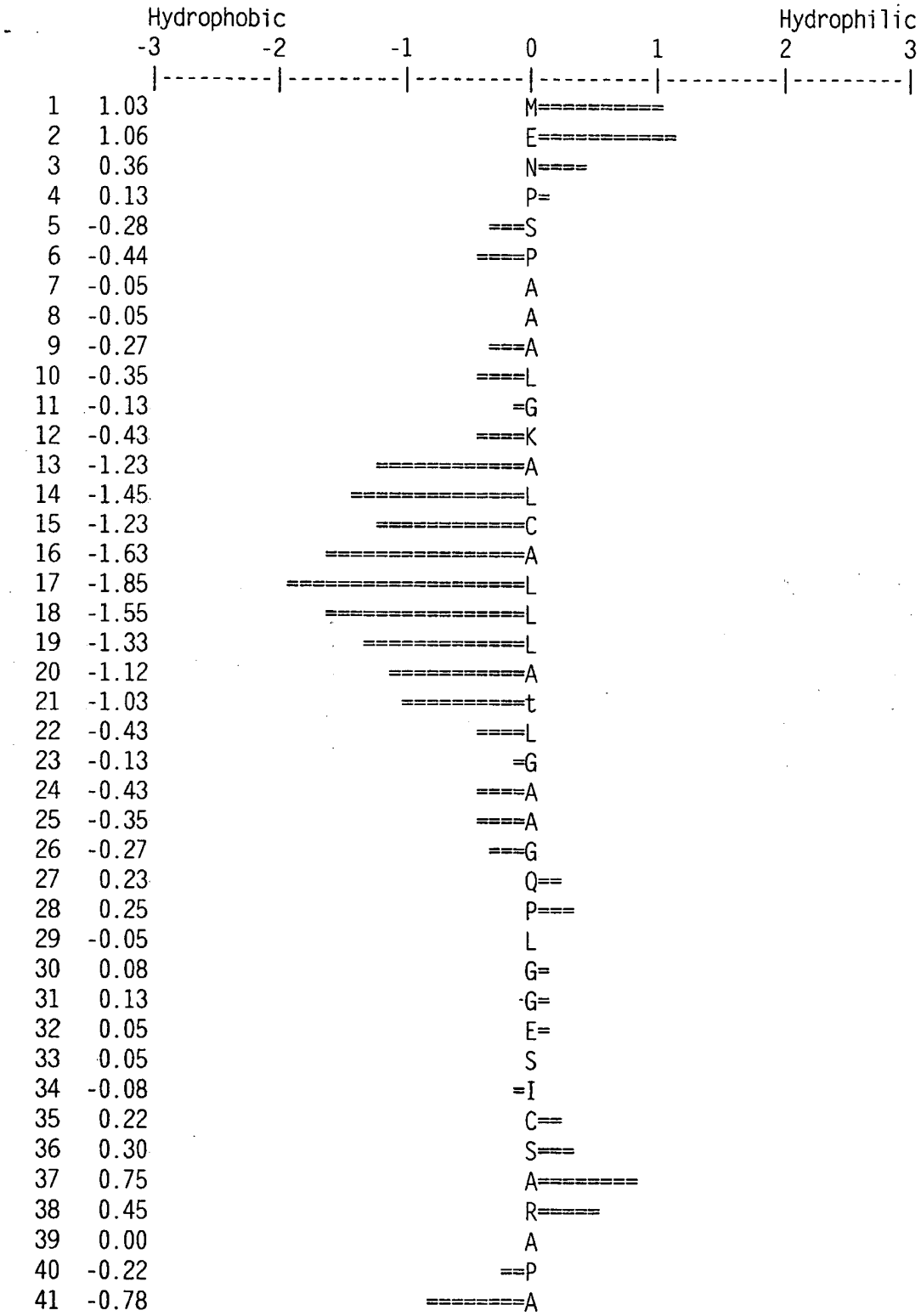
Hydrophobic
Hydrophilic
-3
-2
-1
0
1
2
3
1 1.03 M==========
2 1.06 E==========
3 0.36 N====
4 0.13 P=
5 -0.28 ===S
6 -0.44 ====P
7 -0.05 A
8 -0.05 A
9 -0.27 ===A
10 -0.35 ====L
11 -0.13 =G
12 -0.43 ====K
13 -1.23 ==========A
14 -1.45 ==========L
15 -1.23 ==========C
16 -1.63 ==========A
17 -1.85 ==========L
18 -1.55 ==========L
19 -1.33 ==========L
20 -1.12 ==========A
21 -1.03 ==========t
22 -0.43 ====L
23 -0.13 =G
24 -0.43 ====A
25 -0.35 ====A
26 -0.27 ===G
27 0.23 Q==
28 0.25 P===
29 -0.05 L
30 0.08 G=
31 0.13 G=
32 0.05 E=
33 0.05 S
34 -0.08 =I
35 0.22 C==
36 0.30 S===
37 0.75 A========
38 0.45 R=====
39 0.00 A
40 -0.22 ==P
41 -0.78 ========A

Fig. 8a

```
42  -1.12                    ==========K
43  -1.68                ================Y
44  -1.30                  ============S
45  -0.85                     ========I
46  -0.72                     =======t
47  -0.10                         =F
48   0.35                          T====
49   0.35                          G====
50   0.27                          K===
51  -0.65                     =======w
52  -0.48                      =====S
53  -0.03                          Q
54  -0.03                          T
55   0.05                          A=
56   0.13                          F=
57   0.25                          P===
58  -0.17                        ==K
59  -0.17                        ==Q
60  -0.20                        ==y
61  -0.22                        ==P
62  -0.30                       ===L
63   0.03                         F
64  -0.12                        =R
65  -0.57                     ======P
66  -0.52                      =====P
67  -0.82                    ========A
68  -1.03                  ==========Q
69  -1.63              ================W
70  -1.15                ============S
71  -1.28                ============S
72  -1.42              ==============L
73  -1.07                ==========L
74  -0.72                   =======g
75   0.35                        A====
76   0.45                        A=====
77   0.58                        H======
78   0.45                        S=====
79  -0.17                      ==S
80   0.28                        D===
81   0.28                        y===
82   0.30                        S===
83   0.28                        M===
84   0.12                        W=
85   0.43                        R====
86  -0.02                        K
87  -0.48                      =====N
```

Fig. 8b

88	-0.52	=====Q
89	-0.85	========Y
90	0.03	V
91	0.78	S========
92	0.32	N===
93	0.20	G==
94	0.70	L=======
95	1.50	R===============
96	1.00	D==========
97	1.00	F==========
98	1.33	A============
99	0.85	E==========
100	0.27	R===
101	-0.53	=====G
102	-0.75	========E
103	-0.75	========A
104	-0.17	==W
105	0.10	A=
106	0.68	L=======
107	0.90	M==========
108	1.03	K==========
109	0.53	E=====
110	0.53	I=====
111	0.75	E========
112	-0.05	A
113	0.07	A=
114	0.20	G==
115	-0.05	E
116	-0.63	======A
117	-0.05	L
118	0.00	Q
119	-0.45	=====S
120	-0.45	=====V
121	-0.28	===H
122	-0.20	==E
123	-0.78	========V
124	-0.78	========F
125	-0.37	====S
126	-0.37	====A
127	-0.28	===P
128	-0.35	====A
129	-0.27	===V
130	0.02	P
131	-0.05	=S
132	-0.05	=G
133	-0.13	=T

Fig. 8c

```
134   0.43                                  G====
135   0.13                                  Q=
136   0.60                                  T======
137   0.42                                  S====
138   0.40                                  A====
139   0.98                                  E==========
140   0.98                                  L==========
141   1.60                                  E================
142   1.15                                  V===========
143   1.10                                  Q===========
144   0.82                                  R========
145  -0.25                                ==R
146  -1.17                      ===========h
147  -1.73                   ================S
148  -2.03                ==================L
149  -1.23                      ===========V
150  -1.28                     ============s
151  -0.97                       ==========F
152  -0.55                          ======V
153  -0.25                            ===V
154   0.00                              R
155   0.00                              I
156  -0.27                            ===V
157  -0.43                           ====P
158  -0.68                        =======S
159  -0.73                        =======P
160  -0.98                       ==========D
161  -0.98                       ==========W
162  -0.37                           ====F
163  -0.25                            ===V
164   0.50                              G=====
165   0.20                              V==
166   0.28                              D===
167   0.28                              S===
168   0.23                              L==
169   1.03                              D==========
170   1.03                              L==========
171   1.17                              C===========
172   1.83                              D=================
173   1.83                              G=================
174   1.87                              D==================
175   1.28                              R============
176   0.70                              W=======
177   0.57                              R======
178   0.57                              E======
179  -0.23                            ==Q
```

Fig. 8d

180	-0.65	=======A
181	-0.57	======A
182	-0.87	=========L
183	-0.07	=D
184	-0.65	======L
185	-0.35	====Y
186	-0.03	P
187	0.47	Y=====
188	0.90	D=========
189	0.40	A====
190	0.07	G=
191	-0.50	=====T
192	-0.85	=========D
193	-1.30	============S
194	-1.30	============G
195	-1.30	============F
196	-0.85	=========t
197	-0.70	=======F
198	-0.37	====S
199	-0.48	=====S
200	-0.83	========P
201	-0.83	========N
202	-0.83	========F
203	0.08	A=
204	0.10	T=
205	-0.08	=I
206	0.15	P=
207	0.65	Q=======
208	0.32	D===
209	-0.25	==T
210	-0.13	=V
211	0.17	T==
212	0.28	E===
213	-0.22	==I
214	0.13	T=
215	0.52	S=====
216	0.47	S=====
217	0.33	S===
218	0.32	P===
219	0.37	S====
220	-0.10	=h
221	-0.80	========P
222	-1.18	============A
223	-1.10	===========N
224	-0.63	======S
225	-0.98	==========F

Fig. 8e

226	-0.07	=Y
227	0.23	Y==
228	0.32	P===
229	0.32	R===
230	-0.18	==L
231	-0.18	==K
232	-0.77	========A
233	-0.18	==L
234	-0.13	=P
235	-0.20	==P
236	-0.50	=====I
237	-0.50	=====A
238	0.08	R=
239	-0.72	=======V
240	0.03	T
241	0.13	L=
242	0.48	L=====
243	0.78	R========
244	0.78	L========
245	1.00	R==========
246	0.08	Q=
247	-0.25	===S
248	-0.30	===P
249	-0.30	===R
250	-0.88	=========A
251	-0.80	========F
252	-0.63	======I
253	-0.63	======P
254	-0.63	======P
255	-0.58	======A
256	0.00	P
257	0.50	V=====
258	0.78	L========
259	1.58	P===============
260	1.28	S============
261	0.98	R==========
262	0.98	D==========
263	0.53	N=====
264	0.42	E====
265	-0.03	I
266	0.02	V
267	0.27	D===
268	0.27	S===
269	0.15	A=
270	0.23	S==
271	-0.12	=V

Fig. 8f

272	0.63	P======
273	0.47	E=====
274	0.47	T=====
275	0.28	P===
276	0.33	L===
277	0.33	D===
278	-0.73	=======C
279	-0.52	=====E
280	-0.97	==========V
281	-0.88	=========S
282	-0.93	=========L
283	-0.93	=========W
284	-0.53	=====S
285	-0.58	======S
286	-0.63	======W
287	-0.55	======G
288	-0.72	=======L
289	-0.42	====C
290	0.25	G===
291	-0.05	G
292	-0.05	H
293	-0.03	C
294	0.63	G======
295	0.68	R=======
296	0.68	L=======
297	0.92	G=========
298	1.42	T==============
299	1.10	K==========
300	0.35	S===
301	0.80	R========
302	0.05	T
303	0.15	R==
304	-0.35	====Y
305	-0.05	=V
306	0.23	R==
307	-0.23	==V
308	0.02	Q
309	0.03	P
310	0.03	A
311	-0.05	N
312	-0.08	=N
313	0.38	G====
314	0.08	S=
315	0.53	P=====
316	1.03	C==========
317	1.70	P================

Fig. 8g

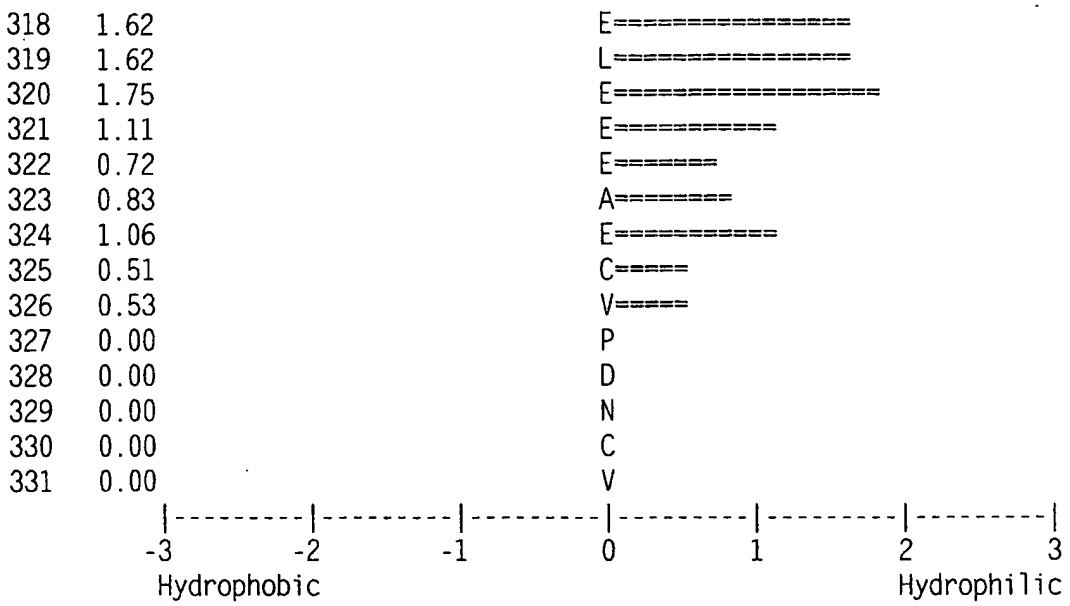
318 1.62 E==========
319 1.62 L==========
320 1.75 E==========
321 1.11 E==========
322 0.72 E=======
323 0.83 A========
324 1.06 E==========
325 0.51 C=====
326 0.53 V=====
327 0.00 P
328 0.00 D
329 0.00 N
330 0.00 C
331 0.00 V
-3 -2 -1 0 1 2 3
Hydrophobic
Hydrophilic

Fig. 8h